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Magnolia : POLYPLOIDY, GENOME SIZE, AND REFINEMENT OF PROTOCOLS FOR MICROPROPAGATION

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ABSTRACT

The genus *Magnolia* includes over 250 species that range in ploidy level from diploid to hexaploid. Although there is basic information on ploidy levels of various species, sampling is limited with specific cultivars and hybrids. The objective of this research was to determine relative genome sizes and relationships to ploidy levels among a diverse collection of species, hybrids, and cultivars using flow cytometry. Nuclei were extracted, stained with 4', 6-diamidino-2-phenylindole (DAPI), and analyzed using a flow cytometer. Relative genome sizes were determined using *Pisum sativum* as the reference genome. Genome size was calibrated with ploidy level for species with documented chromosome numbers. Relative genome size for a given ploidy level varied significantly among most taxonomic sections, indicating it is desirable to calibrate ploidy level with relative genome size for each section separately. Within a section, relative 2C genome sizes, for a given ploidy level, had narrow ranges and could be used to distinguish between euploid levels. Genome size estimates, determined with DAPI or propidium iodide (PI) fluorochromes, varied (by 0% to 14%) as a function of species and base pair composition. Both methods were suitable for determining euploid level. Base pair composition of representative *Magnolia* spp. ranged from 61.6% to 63.91% AT. The results provide insights into reproductive biology, substantiation of hybrids and induced polyploids, and comparison of methods for determining genome size that will facilitate the development of improved hybrids in the future.

Growth responses to basal salt composition, cytokinins, and phenolic binding agents were investigated in a series of experiments to refine *in vitro* culture protocols for *Magnolia* ‘Ann’ for micropropagation and plant improvement applications. Murashige and Skoog basal medium (MS), supplemented with 2 µM benzylamino purine (BAP) with no phenolic binding agent (PBA) generated a 3.2× multiplication rate. Media containing activated charcoal (AC) produced elongated microcuttings more suitable for rooting and *ex vitro* establishment, but AC reduced *in vitro* shoot proliferation. However, during subsequent rooting, microcuttings supplemented with AC *in vitro* had higher *ex vitro* rooting, compared to those without AC regardless of *in vitro* indolebutyric acid (IBA) concentration. Plants subcultured on ½ MS media containing 1g/L AC resulted in acceptable rooting percentages, lateral root development, leaf production, and overall plant appearance and vigor during *ex vitro* establishment.

DEDICATION

I would like to dedicate this work to my family. They have endured this portion of my educational journey with more grace than I could have ever hoped. To my wife Melissa, thank you for holding our home together during my commutes to attend classes, perform research, and adventure far from home. Your patience and prayers during my travels are certainly what brought me safely home each time. To my daughters, Katie and Sarah, thank you for understanding the times that I could not be there. It is my hope that my efforts will be an example that all things are possible through faith, patience, and hard work. To my father and stepmother, Keith and Louise Parris, and my father-in-law and mother-in-law, Richard and Bertice Robinson, thank you for your encouragement and being much more than grandparents to Katie and Sarah. Thanks to you all, and my mother, the late Kay Parris, I have had a family full of educators who have set standards in a noble profession. I suppose it was inevitable that I would settle into this career in education. I may not have done things in the most efficient order, but I cannot imagine a more inspiring or rewarding path.

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Bill and I now know what has driven the likes of these gentlemen and so many others to keep pushing the boundaries of magnolia diversity.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

CHAPTER ONE

INTRODUCTION

Background

The Genus Magnolia is revered throughout the horticultural world for the beauty and interest it lends to its natural habitat, gardens, and commercial landscapes. In 1980 The Journal of the American Magnolia Society published the following comment from E.H. Wilson, the great 19th century British plant explorer: "No group of trees and shrubs is more favorably known or more highly appreciated in gardens than magnolias, and no group produces larger or more abundant blossoms." (Wilson, 1980). In addition to the ornamental merits of magnolias, certain species are valued for timber, food, and medicinal use. The distribution of plants in the family Magnoliaceae also lends significant data used in the studies of plant evolution and biogeography (Cicuzza et al., 2007). Thirteen municipalities in the United States bear the name Magnolia (Wikipedia, 2008). In the Southeastern United States we are most familiar with the evergreen, white tepaled species, Southern Magnolia, *Magnolia grandiflora.* It is a tree synonymous with southern culture. It has garnered so much respect that it has been designated as the state flower of both Louisiana and Mississippi (USNA, 2006). The aristocratic level achieved in gardens, and the utilitarian benefits to populations around the globe, stimulates scientific interest in this genus.

The distribution of Magnolia species worldwide includes populations in Eastern North America, Central America, South America, islands of the Gulf of Mexico, and Eastern Asia (Cicuzza et al., 2007). Fossils have been found that demonstrate the range of

the genera once spread across the North American continent. In fact, a well preserved un-mineralized leaf dating back to the Miocene Era was discovered in Northern Idaho (Weiss, 1990). Glaciation and continental drift are offered as explanations of why the North American and Asian populations are now disjunct. Numerous plant families demonstrate this geographical distribution pattern (Hui-Lin Li, 1952). Taxonomy of magnolia has changed throughout the years: Within the plant family Magnoliaceae there were eleven named genera. Western taxonomists now recognize only two genera; *Magnolia* and *Liriodendron*. The genus *Magnolia* contains many species belonging to various sections within two subgenera, and several others in a third subgenus. The formerly recognized genera were either given sectional status or absorbed into existing sections based on morphological characteristics and molecular systematics (Figlar, 2004). The genus Liriodendron contains 2 species, *Liriodendron tulipifera* occurs in Eastern North America; while *Liriodendron chinensis*, occurs in China. The discovery of *L. chinense* in 1873 was astonishing, as it was previously believed that *L. tulipifera* was a monotypic genus (Hui-Lin Li, 1952). Magnolia and Liriodendron have small genome sizes implying they evolved earlier in the evolutionary timeline than many other angiosperms (Soltis, et al., 2003). The theory that Magnolias are some of the earliest angiosperms is also supported by specialization of floral biology that is preferential to beetle species as pollinators, rather than bees and moths that have a close pollination relationships with flowering plants that evolved much later (Thien, 1974). Realization that the members of the family Magnoliaceae are more closely allied than previously

thought has given Magnolia breeders new hope in the potential for creating more diverse hybrids.

History of magnolia hybridization

Magnolia breeding has been a passion for many enthusiasts since the early 19th century. A brief history of breeding efforts is presented below because the successes and failures revealed why in depth understanding of the magnolia genome is necessary. *Magnolia ×soulangeana* was the first hybrid magnolia of garden origin ever named. It is the result of a cross between *M. denudata*, 2n=6x=114 and *M. liliiflora*, 2n=4x=76. Potentially, this cross would most likely result in a sterile pentaploid, 2n=5x=95, but this has not always been the case. Since the time of the original selection, the cross has been repeated many times and several cultivars have demonstrated some limited fertility although the offspring have odd ploidy levels. Within the *M. ×soulangeana* complex, aneuploids are likely, but because the ploidy level is high, there may be ample genetic redundancy to compensate for any mismatched parings or deletions (Kehr, 1985). Ploidy levels as high as 2n=9x=171 have been estimated in this hybrid complex lending credibility to the theory that unreduced gametes can occur in magnolia. From *M. ×soulangeana* to present day crosses, polyploidy has been a major obstacle in magnolia crossing schemes.

The USNA has been a leader in ornamental plant breeding with introductions of cultivars from the genera that include; *Camellia*, *Ilex, Lagerstroemia*, *Pyracantha*, *Ulmus*, *Viburnum*, and of course, *Magnolia*. Fourteen magnolia cultivars have been

introduced by the USNA (USNA, 2008). Thirteen of these are interspecific hybrids that demonstrate the cross-ability of two species with differing ploidy levels. In 1937 Oliver Freeman made crosses of *M. virginiana* 2n=2x=38 and *M. grandiflora* 2n=6x=114. The resulting tetraploid 2n=4x=76 hybrids include cultivars 'Maryland' and 'Freeman' which were selected from the initial cross. An important aspect of this cross is the hybrids do not display obvious resemblance to the *M. virginiana* parent. This might be due to a complement of chromosomes that is three sets from *M. grandiflora* to one set from *M. viriginiana*. Joseph C. McDaniel, University of Illinois, performed backcrosses of these hybrids with each parent. He found the F_1 hybrids do possess fertility, being able to produce both viable pollen and egg cells. As expected the backcross with *M. grandiflora* results in a pentaploid plant that once again closely resembles *M. grandiflora* phenotype. With this higher ploidy level, McDaniel (1970) also anticipated that the pentaploid could readily produce fertile gametes. This example is reminiscent of the hybrid complex that has developed in *M.* ×*soulangeana* discussed above. When backcrossed with the *M. virginiana* parent, the triploid offspring do begin to resemble *M. virginiana*, since the chromosome complements are now more balanced. Unfortunately these plants are typically sterile, creating a road block for a breeding program (McDaniel, 1970).

The most recognized magnolia introductions from the USNA are the "Little Girl Hybrids", which are the result of crossing *M. liliiflora*, 2n=4x=76 and *M. stellata*, 2n=2x=38. These eight hybrid cultivars, developed by Dr. Francis deVos and Dr. William Kosar are phenotypically intermediate between the parents with the chromosome count 2n=3x=57. Being triploid, these hybrids are sterile. They are easily rooted from

cuttings and have become popular and widely grown by nurseryman around the world. The cultivars ‘Jane’, ‘Betty’, and ‘Ann’ have become particularly well known. They combine the improved characteristics of hardiness, later flowering, and mildew resistance from their parents (Callaway, 1994).

Another pair of hybrid cultivars developed at the USNA by Dr. William Kosar are the result of crossing *M. liliiflora* ‘Nigra’, 2n=4x=76 and *M. sprengeri* ‘Diva’, 2n=6x=114. The resulting hybrid cultivars are also popular in the nursery and landscape industry. These pentaploids, 2n=5x=95, known as ‘Galaxy’ and ‘Spectrum’, have symmetrical upright growth habits and later flowering that allow them to avoid late spring frosts. Dr. Kosar also developed the hybrid *M.* x ‘Nimbus’ by crossing *M. virginiana* and *M. obovata*, both diploids (Callaway, 1994).

In 1954 a milestone cross was made by breeders at the Brooklyn Botanic Garden, Brooklyn, NY between *M. acuminata* and *M. liliiflora*. Each parent species is 2n=4x=76. This cross has resulted in a number of fertile cultivar introductions, including ‘Woodsman’, and ‘Evamaria’. Another breakthrough was achieved at Brooklyn Botanic Garden in 1956 when *M. acuminata* 2n=4x=76 was crossed with *M. denudata* 2n=6x=114, leading to the introduction of M. ‘Elizabeth’. This hybrid is a pentaploid and partially fertile (Callaway, 1994). These were both significant crosses because they opened the door for breeders like Phil Savage, Bloomfield Hills, MI, August Kehr, Hendersonville, NC, Dennis Ledvina, Green Bay, WI, and Bill Smith, Richmond, VA to continue to work with fertile hybrid offspring, selections of *M. acuminata*, and cultivars from the *M.* ×*soulangeana* hybrid complex utilizing inter and intra ploidy crosses.

Todd Gresham was another prolific breeder who was able to take advantage of the favorable environment in Santa Cruz, CA to maintain hybrids involving *M.* ×*veitchii* (which incorporates *M. campbellii*), *M.* ×*soulangeana, and M. liliiflora* and to generate over 15,000 hybrid plants. New Zealand breeders Oswald Blumhardt and Felix Jury are also known for their hybrids involving these species. Frank Galyon of Knoxville, Tennessee also obtained successful F1 progeny from crosses between *M. stellata, M. denudata, M. liliiflora,* and *M. sprengeri* (Callaway 1994).

While the above successes in hybridization show reproductive compatibility between species in different sections and ploidy levels, they are primarily within Subgenus Yulania which has not had encouraging results when crossed with species from Subgenus Magnolia. While researchers prefer to publish results that promote successes, it is also important to document failure. In 1979 USNA research geneticist Frank Santamour Jr. reported the crosses between *M. viriginiana* (Subgenus Magnolia) and ten magnolia taxa from Subgenus Yulania. The possibility of introgression of flower color in these crosses, if successful, would be likely since the higher ploidy levels of the colored tepal pollen parent from Subgenus Yulania would outweigh the contribution of the diploid *M. virginiana*. A total of 179 crosses were made with no seed produced. Santamour did not rule out the possibility of intersubgeneric crosses involving *M. virginiana,* but wanted to illustrate that hybrids from this cross would not be easily obtained. The other important statement made by Santamour (1979) was a reference to successful but yet unpublished results from a crossing of *M. grandiflora* and *M. liliifora* (Santamour, 1979).

From two crosses made in 1971 of *M. grandiflora*, 2n=6x=114 and *M. acuminata var. subcordata*, 2n=4x=76, 34 seed was obtained and two seedlings were successfully germinated. *M. acuminata var. subcordata* has yellow carotenoid pigments. (Santamour, 1979a). Cytological studies of these accessions MAG 14-1 and MAG 17-1, documented 2n=5x=95, confirming the true hybridity of these plants, making them the first reported intersubgeneric hybrids within Magnolia. MAG 17-1, a deciduous plant, died following winter injury in 1978. MAG 14-1, with evergreen foliage, survived, suggesting cold hardiness was transmitted from *M. acuminata var. subcordata* to a hybrid with *M. grandiflora*. MAG 14-1 flowered, but did not show significant introgression of yellow flower color from the pollen parent. Santamour (1979a) suggested the reciprocal cross maybe successful for generating yellow-flowered evergreen hybrids due to maternal inheritance of plastids. Confirmation of this hypothesis was not successful, because all reciprocal crosses failed (no hybrid seeds). Evidence provided by Sewell, et al., (1993) suggests that plastid inheritance in the genus Magnolia may be more complex. Sewell, et al. (1993) demonstrated traces of paternal transmission of plastids in *Liriodendron* and *Magnolia sp*.

The most exciting report about intersubgeneric hybridization was provided by Santamour (1979). Crosses were made in 1969 between *M. grandiflora* and the purple tepaled *M. liliflora.* The author assumed the likelihood of flower color being introgressed from the pollen parent would be greater in this cross due to nuclear inheritance of anthocyanin genes. Santamour (1979) collected thirty-eight seed from crosses on two trees. Two other trees failed to produce any mature fruit from the crosses. One seedling

showed characteristics of hybridity and was later confirmed to be 2n=5x=95 by a chromosome count. The nineteen remaining seedlings from that cross, which visually could not be discerned from typical *M. grandiflora* seedlings, were also analyzed and ten of these were found to be pentaploid hybrids. Ten seedlings raised from the other cross were all found to be 2n=6x=114, just like the maternal parent. Santamour (1979), states that apomictic seed is well known in *M. grandiflora,* explaining the non-hybrids. In regard to flower color, five of the ten hybrids had flowered by 1980, all having white tepals. Santamour (1979) acknowledges the breeder's objective of introducing a red to pink flowered evergreen magnolia has not been realized using the F_1 generation, but proposes backcrossing onto the deciduous parent with pigmented flowers. Santamour (1979) acknowledges that this would decrease the likelihood of that offspring being evergreen. He also reports success rooting the putative *M. grandiflora* × *M. liliflora* hybrids and distribution to cooperators in 1981 (Santamour, 1981).

The breeding efforts with Magnolia throughout the twentieth century have mostly been focused within Subgenus Yulania. This would include all of the *M. ×soulangeana* cultivars, the "Little Girl" hybrids, and the yellow flowered cultivars derived from *M. acuminata.* There has been less effort with Subgenus Magnolia, though hybrids have been produced between *M. virginiana* and the following species; *M. obovata, M. tripetala, M. macrophylla,* and recently, *M. insignis, M. yuyuanensis, and M. sieboldii.* Except for the mention of the USNA work with intersubgeneric hybridization, no well documented efforts have been focused in that direction. The significant hurdle in overcoming difficult breeding obstacles may be the time factor that derails the breeders

from their objectives (Seidl, 1983), and genetic and reproductive barriers, which ultimately could be impossible to overcome using conventional technologies.

Several avenues might be explored that could lead to new magnolia cultivars, especially evergreen or ultimately yield specimens that possess pink flowers and *M. grandiflora*-like evergreen foliage. If *M. grandiflora* owes its hexaploid condition to accumulation of introgressed genes from its closely related species (McDaniel, 1970) then we should investigate the reproductive compatibility within Subgenus Magnolia, such as Section Manglietia. Germplasm from various taxa, now readily accessible, may provide resources for new breeding objectives. The studies documented in this thesis have been conducted with the goal that they might be useful in the future to clarify reproductive barriers and facilitate new opportunities for breeders of magnolia.

CHAPTER TWO

PLOIDY LEVELS, RELATIVE GENOME SIZES, AND BASE PAIR COMPARISON IN MAGNOLIA

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Introduction

Polyploidy has been an important process in the evolution of plants that can contribute to reproductive isolation, novel gene expression, and ultimately divergence and speciation (Adams and Wendel, 2005; Comai, 2005; Hegarty and Hiscock, 2008; Soltis et al., 2003; Soltis and Burleigh, 2009). Polyploidy is also an important factor in plant breeding as it can influence reproductive compatibility, fertility, and phenotypic traits (Chen and Ni, 2006; Jones and Ranney, 2009; Ranney, 2006; Soltis et al., 2004). In some cases, the artificial induction of polyploidy in *Magnolia* also can enhance ornamental characteristics including thicker leaves and larger flowers with thicker petals

that persist longer (Kehr, 1985). As such, accurate and specific knowledge of ploidy levels of species and cultivars is important information for magnolia breeders.

The genus *Magnolia* comprises more than 250 species belonging to various sections within three subgenera (Figlar and Nooteboom, 2004). Although basic information on chromosome counts and ploidy levels of different *Magnolia* sp. have been compiled (Callaway, 1994; Chen, et al., 2000), sampling has been limited and little is known about ploidy levels of specific hybrids and cultivars. The base chromosome number for *Magnolia* is $1n = 1x = 19$. However, different subgenera contain species with a variety of ploidy levels ranging from $2n = 2x = 38$ to $2n = 6x = 114$. Crosses between species with varying ploidy levels may yield hybrids with nonstandard chromosome numbers that can result in reduced fertility or sterility. Because of these constraints, *Magnolia* breeders have attempted to induce new polyploids to overcome these limitations, yet most of these putative polyploids have never been confirmed. The range in ploidy levels within this genus also provides an opportunity to indirectly substantiate hybridity, when parents differ in ploidy levels.

Since many *Magnolia* species are polyploids with high chromosome numbers, traditional cytology based upon light microscopic examination is a difficult and time consuming process. Flow cytometry has proved to be an efficient means of estimating genome size and associated ploidy level (Doležel et al., 2007; Jones et al., 2007). Therefore, the objectives of this study were to determine the genome sizes and relationships to ploidy levels of a diverse collection of species, hybrids, and cultivars of *Magnolia* to 1) to develop an extensive database of ploidy levels for use by magnolia

breeders, 2) determine the ploidy levels of plants that were chemically treated to artificially induce polyploidy, 3) confirm hybridity of interploid and interspecific (when parents vary substantially in genome size) crosses, and 4) compare estimates of genome size using DAPI (AT preferential) or PI (intercalating) fluorochrome stains and estimate base pair composition for representative taxa from 10 taxonomic sections.

Materials and Methods

Relative genome size and ploidy level determination. Over 300 accessions were sampled from various sources that included 62 species, 125 hybrids, and 16 induced polyploids representing taxa from each subgenus of *Magnolia* as well as both species of *Liriodendron*, the only other genus in family Magnoliaceae per Figlar and Nooteboom (2004). Nuclei from newly expanded leaf or tepal tissue were extracted, stained with DAPI, and then analyzed (minimum of 2500 nuclei per sample) using a flow cytometer (PA-I; Partec, Münster, Germany) to determine relative holoploid 2C DNA content following the methods of Jones et al. (2007). Genome sizes were determined by comparing mean relative fluorescence of each sample with an internal standard, *P. sativum* 'Ctirad', with a known genome size of 8.76 pg (Greilhuber et al., 2007). Because tetraploid *Magnolia* taxa have similar genome sizes to *P. sativum* 'Ctirad', *Magnolia virginiana* 'Jim Wilson' (NCSU 2004-24) (3.92 pg) was used as a secondary standard. Absolute genome size for the secondary standard was calculated as the mean of 10 separate subsamples determined with *P. sativum* 'Ctirad' as an internal standard and propidium iodide (PI) as the fluorochrome stain (see procedure below in *Comparison of*

fluorochromes and estimate of base pair composition). Holoploid, 2C DNA contents were calculated as: 2C = DNA content of standard × (mean fluorescence value of sample ÷ mean fluorescence value of the standard).

The relationship between ploidy levels and genome sizes was determined for plants with documented chromosome numbers (Chen et al., 2000). Mean 1Cx monoploid genome size (i.e., DNA content of the non-replicated base set of chromosomes with x = 19) was calculated as 2C genome size ÷ ploidy level, to assess variability in base genome size. A minimum of two subsamples were tested to derive a mean relative genome size for each accession. Data for species were subjected to analysis of variance and means separation using the Waller procedure (Proc GLM, SAS version 9.1; SAS Institute, Cary, NC). Ploidy levels for hybrid taxa and suspected aneuploid hybrids were derived in the following manner: ploidy level = mean 2C genome size ÷ weighted average 1Cx genome size of the reported parental species.

Comparison of fluorochromes and estimate of base pair composition. Ten species were sampled that included taxa from each subgenus of *Magnolia*. Nuclei were extracted, stained, and analyzed as described previously using a minimum of 3000 nuclei per sample. Sample preparation was similar to methods described for DAPI, with the exception that the staining solution consisted of 2 mL staining buffer, 6 µL RNase A, and 12 µL PI (CyStain PI absolute P, Partec) and the samples were maintained at 4 °C for 1h before flow cytometry analysis using a 488-nm laser for excitation (PA-II; Partec). The experimental design was a split-plot design with fluorochrome (DAPI vs. PI) as the whole plot and species as the sub-plot. Samples were collected and analyzed over time in

complete blocks. Data were subjected to analysis of variance and mean separation using Fisher's least significant difference ($LSD_{0.05}$) specifically calculated for comparing two whole plot (fluorochrome) factors for a given subplot (species). Base pair composition was calculated following the equation: AT% = AT% for internal standard × [(fluorescence internal standard, DAPI/fluorescence sample, DAPI) ÷ (fluorescence internal standard, PI/fluorescence sample, PI)]$^{(1/\text{binding length})}$ (Godelle, et al., 1993), where AT% of the internal standard, *Pisum sativum,* = 61.50% and binding length of DAPI ~3.5 bp (Meister and Barow, 2007).

Cytology. Actively growing root tips of container grown seedlings of putative octaploid *M. cylindrica* were collected at midday and placed in the mitotic inhibitor, 8-hydroxyquinoline for 2 h at 5 °C in dark conditions. They were then transferred to a fixative solution of 3 parts 95% ethanol: 1 part glacial acetic acid (v/v) for 24 h, while remaining at 5 °C in dark conditions. Tissue was excised from just behind the root tip and placed in 12N HCl for 10 s. Squashes were prepared with a small amount of this tissue and a drop of modified Fuelgen stain on a slide with a cover slip.

Results and Discussion

Relative genome size and ploidy level among species. Relative genome sizes and ploidy levels were determined for 175 accessions, representing 62 species of Magnoliaceae and arranged by taxonomic sections following Figlar and Nooteboom (2004) (Tables 1 and 2). Base, 1Cx genome size varied significantly among plants sampled from different taxonomic sections indicating these groups have undergone

considerable genome size divergence (Table 1). This variation indicates it is necessary to calibrate ploidy level with genome size for each section to estimate ploidy level from genome size in *Magnolia*. However, within a section, genome sizes for a given ploidy level had sufficiently narrow ranges that they could be used to clearly determine ploidy levels. Diploidy was prevalent throughout taxonomic sections, but variation in ploidy level occurred among species within several sections. Section *Magnolia* in subgenus *Magnolia* had both diploid and hexaploid members while section *Yulania* in subgenus *Yulania* was represented by diploid, tetraploid, and hexaploid species. The two species tested in section *Gynopodium*, subgenus *Gynopodium*, were both hexaploid

Ploidy levels of species were generally consistent with past reports (Chen et al., 2000; Treseder, 1978; Xia et al., 2008), with some new additions and clarifications. Samples from wild collected *M. cylindrica* (Bartlett 193; Holden 96-111A; Holden 96-115B; and MGA 216/Holden 87-86-93) were found to be tetraploid, having relative 2C genome sizes ranging from 8.82 to 9.11 (Table 2), in agreement with Xia et al. (2008), but not with prior reports (Treseder, 1978) that indicated *M. cylindrica* was diploid. Earlier reports may have varied due to lack of confirmed, wild collected accessions in gardens of Europe and North America as stated by Callaway (1994). Chromosome counts have not been published for *M. zenii*, a species recently introduced into cultivation. The three accessions of *M. zenii* (MGA 440/ Arnold 1545-80-B, Chollipo Form, and 'Pink Parchment') tested here were diploid with a mean relative genome size of 4.16 pg. *Magnolia biondii* has been reported to be tetraploid (Xia et al., 2008), though we found two *M. biondii* accessions (MGA 027 and Bartlett 2002-056) to be diploid with

a mean relative genome size of 4.11 pg. In our study, no natural variation in ploidy level was found among accessions within a given species.

Relative genome size and ploidy level among hybrids. Genome sizes and ploidy levels were determined for a broad range of reported interspecific, intra and interploid hybrids (Table 3). In certain cases, analysis of genome size helped to substantiate or refute the authenticity of the hybrids. For example, the intersectional, intraploid hybrid *Magnolia* 'Katie-O' (NCSU 2004-012, MGA 307) had a mean 2C genome size of 4.30 pg, intermediate between the reported parents of *M. insignis* (2C = 4.94 pg) × *M. virginiana* (2C = 3.72 pg), supporting hybridity. Additional interspecific, intraploid hybrids strongly supported by genome size analysis include *M. yuyuanensis* × *M. virginiana*, NCSU 2009-131; *M. virginiana* 'Havener' × *M. insignis* Red Form, 111/7, McCracken; and ((*M. tripetala* × *M. obovata*) × *M. tripetala)* 'Silk Road' × *M. insignis* (MGA). Flow cytometry did not typically allow for distinguishing interspecific hybrids within a given section and ploidy level due to conserved genome sizes within sections. Taxa including *M.* ×*kewensis*, *M.* ×*loebneri*, *M.* ×*brooklynensis*, and *M.* ×*veitchii* fall into this category.

Evidence for successful hybridization between plants of different ploidy levels was apparent based on analysis of genome sizes. In many cases interploid hybrids were substantiated. These include the following within subgenus *Magnolia*: (*M. grandiflora* (6*x*) × *M. virginiana* (2*x*)) 'Maryland' (MGA 077, McCracken) with an intermediate genome size of 7.49 pg, and also a seedling of 'Maryland'(MGA 325) which was likely open pollinated by *M. grandiflora* that had a genome size of 9.00 pg, consistent with a

pentaploid derived from a tetraploid by hexaploid cross. An unnamed plant at the U.S. National Arboretum (USNA 2) with morphological similarity to *M.* 'Maryland' was found to have a genome size of 5.62 pg, consistent with a triploid, suggesting a *M. grandiflora* (6*x*) x *M. virginiana* (2*x*), backcrossed to *M. virginiana*. An intermediate tetraploid condition was determined for *M. insignis* (2*x*) × *M. grandiflora* 'Kay Parris' (6*x*) (NCSU H2010-026-001), which had an 8.50 pg relative genome size.

Within subgenus *Yulania*, confirmed interploid hybrids were numerous. Verification of hybridity was readily confirmed for the U.S. National Arboretum's Kosar / de Vos hybrids. *M. liliiflora* (4*x*) × *M. stellata* (2*x*) had genome sizes ranging from 6.28 to 6.69 pg, consistent with triploids. Numerous putative pentaploid hybrid cultivars, derived from crosses of (6*x* × 4*x*) species or hybrids, were also verified. These hybrids include: 'Alexandrina', 'Angelica', Apollo', 'Blushing Belle', 'Butterflies', 'Elizabeth', 'Galaxy', 'Gold Finch', and 'Spectrum' with 2C genome sizes ranging from 10.11 to 11.02 pg.

Hybrids arising from parents with odd ploidy levels (5*x* or aneuploids) were prevalent and had highly variable genome sizes. *Magnolia* ×*soulangeana*, a pentaploid hybrid between *M. denudata* (6*x*) and *M. liliiflora* (4*x*) exhibits fertility in initial F_1 hybrids and subsequent generations (McDaniel, 1968), and when used as parents gave rise to apparent aneuploid progeny ranging from ~4.6 to ~8.5*x* based on genome size. Fertility among *M.* ×*soulangeana* cultivars has been examined previously and it was found that pollen viability generally increased with increasing ploidy level above 5*x* (Santamour, 1970). Relative 2C genome sizes determined here support cytological findings by Santamour

(1970) that the cultivars Lennei and Grace McDade are septaploids or higher. Other taxa in Table 3 of approximately septaploid genome size include *M.* 'Andre Leroy' (Milliken), *M.* 'Manchu Fan' (Bartlett 2003-593), *M.* 'Sunsation' (SCC), and *M.* 'Todd Gresham' (Bartlett 2002-641). Each of these hybrids has a parental combination that theoretically could yield 7*x* offspring. No triploid hybrids were found to be parents of any hybrid surveyed in this study indicating triploids may typically not be fertile.

In a number of cases interploid hybridization was not validated. Two accessions of *Magnolia* 'Sweet Summer' (11.53 pg) (McCracken, MGA 327) a reported *M. virginiana* (2*x*) × *M. grandiflora* (6*x*) hybrid, and *M.* 'Monland'(11.29 pg) (SCBG) a reported *M. grandiflora* (6*x*) × *virginiana* (2*x*) hybrid (Langford, 1994), both had genome sizes consistent with a subgenus *Magnolia* hexaploid.

Unreduced gametes can lead to higher than expected genome sizes or ploidy levels in Magnolia hybrids (McDaniel, 1968; Santamour, 1970). In subgenus Yulania, the relative genome size of *M. acuminata* (*4x*) × M. *stellata* (*2x*) 'Gold Star' (NCSU 2004-063) was determined to be 8.22 pg, consistent with the genome size of a tetraploid. This suggests this cultivar is the result of pollination from an unintended source or the product of an unreduced gamete from *M. stellata*. The hybrids 'Miranja' and 'Sunsation' may also have resulted from stray pollination or unreduced gametes from at least one parent.

Determination of relative genome size and ploidy level among artificially induced polyploids. Attempts to develop artificially induced polyploids of *Magnolia* have met with varying degrees of success. *Magnolia stellata* and *M. cylindrica* seedlings treated

with colchicine at the Holden Arboretum (Charles Tubesing, personal communication) were determined to be tetraploid and octoploid, respectively (Table 4). *Magnolia kobus* 'Norman Gould' (7.79 pg) (USNA 59598-H) was also confirmed to be tetraploid. Additonally, a *M. grandiflora* 'Little Gem' treated with colchicine at Head-Lee Nursery (Bob Head, personal communication) was determined to be a 6*x* - 12*x* cytochimera. The plant was reported to be treated over 10 years ago and has stabilized as a cytochimera with approximately 55% of the leaf tissue comprised of 12*x* cells. Phenotypic characteristics such as thickened foliage and increased width to length ratio of foliage (Kehr, 1985) were suggestive of polyploidy in *M. sieboldii* 'Colossus', a reported hexaploid. However, samples of *M. sieboldii* 'Colossus' from multiple sources had genome sizes (2C = 4.35 pg to 4.62 pg) consistent with a diploid. Hybrids with *M.* 'Colossus', including *M. sieboldii* 'Colossus'× *M. grandiflora* 'Bracken's Brown Beauty' (McCracken), *M. sieboldii* 'Colossus'× *M. grandiflora* 'Kay Parris' (KP 2008-001), and *M. sieboldii* 'Colossus'× *M.* 'Sweet Summer' (MGA 280) (Table 3) all had relative genome sizes consistent with a tetraploid, further confirming the ploidy level of the diploid and hexaploid parents. Other reported induced polyploids that were not confirmed include *M. stellata* 'Two Stones' and *M. acuminata* 'Patriot'. Seedlings SCC-2009-004 and SCC-2009-005, derived from open pollinated octoploid *M. cylindrica* at the Holden Arboretum were determined to be approximately 7*x* based on a genome sizes of 14.92 to 15.21 pg. This supports the assertion of Charles Tubesing (personal communication) that the octoploids probably outcrossed with other magnolias with lower ploidy levels from their collections. A chromosome count of one of these seedlings, SCC

2009-004, identified approximately 133 chromosomes (Fig. 1), in close agreement with genome size data.

Comparison of fluorochromes and estimate of base pair composition. Comparison of DAPI and PI stains showed there was a significant interaction between fluorochrome stain and species on the estimation of genome size ($P \leq 0.05$) (Table 5). For some species, e.g., *M. sinica*, *M. stellata* 'Royal Star', and *M. yuyuanensis*, there was no significant difference in genome size estimates between fluorochromes. In other cases, the difference in genome size estimates varied by as much as 0.73 pg or 14% for *M. delavayi*. This suggests that as base pair composition of the sample deviates from the base pair composition of the internal standard (in this case *P. sativum* = 61.50% AT), the estimate of genome sizes between methods diverges. However, for the purpose of determining euploid levels, either method was sufficiently accurate to provide proper classification and the DAPI procedure is faster, less expensive, utilizes less toxic compounds, and can have lower coefficients of variation for mean nuclei fluorescence than the PI procedure. Base pair composition of representative *Magnolia* spp. ranged from 61.6% to 63.9% AT. Sequences of 8500 bases of cpDNA from seven different regions of 43 different species of *Magnolia* showed the relative frequency of AT ranging from 62.9% to 63.1% (H. Azuma, personal communication), similar to the range of that we determined for the entire nuclear genome based on differential fluorochrome staining.

Implications of relative genome size for systematics and breeding. The most recent taxonomic revision of *Magnolia* (Figlar and Nooteboom, 2004) incorporates both

morphological and molecular data (Azuma et al., 1999, 2000, 2001; Kim et al., 2001). In some cases, data on relative genome size support these revised taxonomic groupings. For example, establishment of section *Macrophylla* to include only *M. macrophylla* and botanical varieties *ashei* and *dealbata* is supported by the difference in 1Cx value (Table 2) of this group compared to other North American species (*M. fraseri* and *M. tripetala*) with which it was traditionally grouped (Treseder 1978). However, in other cases there is inconsistent variation in genome size within some sections (e.g., *M. rostrata* in section *Rhytidospermum*) and similarities in genome size among distantly related taxa (Table 2).

Conclusion

For breeders, the revised taxonomy by Figlar and Nooteboom (2004) provides a greater understanding of the relatedness and potential for interspecific hybridizations among closely allied species that is often supported empirically (Table 3). Yet, development of progeny from hybrids, beyond an F_1 generation, requires genome/chromosomal compatibility for meiosis to function properly. Thus, it is reasonable to expect that the greater the difference in genome size among parental species, the less likely hybrid progeny will be fertile.

Results from this study provide data on genome sizes and ploidy levels of a broad range of species and hybrids of *Magnolia*. This information also gives insights into reproductive biology, confirmation of hybrids and induced polyploids, and comparison of methods for determining genome size that will help facilitate the development of improved hybrids in the future.

Table 2.1. Summary of means and ranges for 2C, holoploid genome size (pg) and 1Cx monoploid genome size (pg) of *Magnolia* spp. grouped by section and ploidy level.

	Ploidy level [z]		
Classification	$2n = 2x = 38$	$2n = 4x = 76$	$2n = 6x = 114$
Subgenus Magnolia			
Section Magnolia (5/41[y])	2C = 3.80[x] **E**[w] (3.43 - 4.40) [u] $1C_X = 1.90$[t] (1.72 - 2.20) [s]	N[v]	2C = 11.18 **C** (10.83 - 11.86) $1C_X = 1.86$ (1.81 - 1.98)
Section Gwillimia (4/6)	2C = 5.32 **A** (5.10 - 5.63) $1C_X = 2.66$ (2.41 - 2.82)	N	N
Section Rhytidospermum (5/18)	2C = 4.27 **CD** (3.66 - 4.69) $1C_X = 2.14$ (1.83 - 2.35)	N	N
Section Manglietia (10/17)	2C = 4.87 **B** (4.65 - 5.25) $1C_X = 2.44$ (2.33 - 2.63)	N	N
Section Macrophylla (1/5)	2C = 4.57 **BC** (4.41 - 4.87) $1C_X = 2.28$ (2.21 - 2.44)	N	N

Section Auriculata (1/3)	2C = 3.83 **E** (3.74 - 3.96) $1C_X$ = 1.94 (1.87 - 1.98)	N	N
Section Kmeria (1/1)	2C = 5.51 **A** (5.51 - 5.51) $1C_X$ = 2.76 (2.76 - 2.76)	N	N
Subgenus Yulania			
Section Yulania (14/43)	2C = 4.05 **DE** (3.84 - 4.26) $1C_X$ = 2.02 (1.92 - 2.13)	2C = 8.56 **A** (8.08 - 9.34) $1C_X$ = 2.14 (2.02 - 2.34)	2C = 12.68 **A** (11.49 - 13.47) $1C_X$ = 2.11 (1.92 - 2.25)
Section Michelia (17/31)	2C = 4.56 B**C** (4.23 - 4.92) $1C_X$ = 2.28 (2.11 - 2.46)	N	N
Subgenus Gynopodium			
Section Gynopodium (2/3)	N	N	2C = 11.93 **B** (11.57 - 12.50) $1C_X$ = 1.99 (1.93 - 2.08)
Section Manglietiastrum (1/1)	2C = 4.21 **D** (4.21 - 4.21) $1C_X$ = 2.11 (2.11 - 2.11)	N	N

Genus *Liriodendron* (2/2)	2C = 3.41 **F**	N	N
	(3.35 - 3.47)		
	$1C_X$ = 1.71		
	(1.68 - 1.74)		

[z] Taxa assigned to given ploidy level based on estimated genome sizes and in agreement with published chromosome counts, if available.

[y] Numbers in parentheses, following classifications, indicate the number of species sampled, and the total number of taxa within those species sampled.

[x] Relative 2C genome sizes (pg) were determined using 4',6-diamidino-2-phenylindole as the flourochrome stain.

[w] Letters following Relative 2C genome sizes, within a column, are significantly different, using the Waller Procedure(Proc GLM, SAS version 9.1; SAS Institute, Cary, NC) for means separation, at $P < 0.05$.

[v] N = No genome size reported; indicates given ploidy level was not reported or observed in this section.

[u] Values represent ranges of 2C genome size for all *Magnolia* spp. sampled in each section.

[t] Relative 1Cx mean genome sizes (pg) were calculated as: (2C mean / ploidy level).

[s] Values represent ranges of $1C_X$ genome size means for all *Magnolia* spp. sampled in each section.

Table 2.2. Relative genome size (pg) and estimated ploidy level for a diverse collection of Magnoliaceae representing 62 species.

Taxa	Cultivar/selection	Source/accession no.[z]	Relative 2C genome size (pg) [mean {plus minus} SE (pg)][y]	Mean relative 1Cx genome size by species (pg)[x]	Ploidy level (*x*)
Subgenus Magnolia					
Section Magnolia					
virginiana	NCSU Variegated	Bartlett in nursery	3.51 ± 0.06	1.86	2
	'Northern Belle'	Bartlett 2005-1177A	3.68 ± 0.02		2
	'Plena'	Bartlett 2007-0041	3.67 ± 0.03		2
	R14-397	McCracken	3.73 ± 0.01		2
	SCC Littleleaf	SCC	3.84 ± 0.07		2
virginiana var. *australis*	'Aiken County'	Bartlett 2004-644	3.69 ± 0.12		2
	'Coosa'	MGA 172	3.78 ± 0.06		2
	'Henry Hicks'	Bartlett 2003-603	3.68 ± 0.08		2
	'Jim Wilson'	NCSU 2004-24	3.75 ± 0.03		2
	'Santa Rosa'	Gilbert's Nursery	3.89 ± 0.07		2
	'Silver Savage'	MGA 255	3.71 ± 0.02		2
	'Tensaw'	McCracken	3.73 ± 0.01		2
	Texas/Lousiana Form	Bartlett 2002-269	3.43 ± 0.07		2
grandiflora	'24 Below'	NCSU	11.32 ± 0.03	1.87	6
	'Black Stem'	McCracken	11.18 ± 0.14		6
	'Bracken's Brown Beauty'	Milliken	11.07 ± 0.04		6
	'Carolina Compact'	McCracken	11.04 ± 0.02		6

	‘Charles Dickens’	MGA 353	10.88 ± 0.01		6
	Charles Dickens Seedling	MGA	11.07 ± 0.00		6
	‘Claudia Wannamaker’	Milliken	11.03 ± 0.02		6
	‘Coco’	Forest St./Spartanburg	10.91 ± 0.06		6
	‘D.D. Blanchard’	Gilbert’s Nursery	11.13 ± 0.13		6
	‘Edith Bogue’	Milliken	11.06 ± 0.06		6
	‘Edith Bogue’	McCracken	11.16 ± 0.17		6
	‘Gallisonier’	McCracken	11.47 ± 0.30		6
	‘Harold Poole’	Head	11.64 ± 0.18		6
	‘Kay Parris’	NCSU	11.10 ± 0.09		6
	‘Little Gem’	NCSU 1998-406	11.16 ± 0.11		6
	‘Main Street’	Bartlett 2006-0124A	10.83 ± 0.23		6
	‘MGTIG’ Greenback	Gilbert’s Nursery	11.12 ± 0.17		6
	‘Pat’s Variegated’	Bartlett 2007-0566A	11.06 ± 0.02		6
	‘Phyllis Barrow’	Milliken	11.14 ± 0.06		6
	‘Reigel’	McCracken	11.49 ± 0.06		6
	‘Samuel Sommer’	Strybing	11.86 ± 0.00		6
	‘Scituate’	McCracken	10.98 ± 0.06		6
	‘Smith Fogle’	McCracken	11.49 ± 0.13		6
	‘Southern Charm’	SCC	10.84 ± 0.02		6
	USNA 1	USNA	11.09 ± 0.00		6
	USNA 3	USNA	11.32 ± 0.00		6
guatamalensis		Strybing 1992-0143	4.37 ± 0.02	2.19	2
sharpii		Strybing 1984-0182	4.40 ± 0.04	2.20	2
tamaulipana		MGA 191	11.01 ± 0.08	1.88	6
	‘Bronze Sentinel’	Gilbert’s Nursery	11.63 ± 0.15		6
Section Gwillimia					
Subsection Gwillimia					
coco		MGA in nursery	4.83 ± 0.04	2.42	2

delavayii		MGA 411	5.10 ± 0.05	2.64	2
		Strybing xy-0179	5.46 ± 0.02		2
Subsection Blumiana					
hodgsonii		Strybing	5.47 ± 0.14	2.73	2
		NCSU 2010-084	5.42 ± 0.01		2
liliifera		MGA in nursery	5.63 ± 0.01	2.82	2
Section Rhytidospermum					
Subsection Rhytidospermum					
obovata (hypoleuca)		MGA 179	3.97 ± 0.01	1.99	2
officinalis var. *officinalis*		MGA 471	4.01 ± 0.01	1.89	2
officinalis var. *biloba*		MGA 111	3.78 ± 0.02		2
		Bartlett 2002-196	3.66 ± 0.03		2
		McCracken	3.68 ± 0.03		2
rostrata		NCSU	4.69 ± 0.07	2.35	2
tripetala		SCBG	4.05 ± 0.00	2.00	2
		MGA 135	3.94 ± 0.01		2
Subsection Oyama					
sieboldii	‘Brusso’ seedling	SCC 2008-101	4.41 ± 0.03	2.26	2
	‘Colossus’	NCSU 2004-064	4.62 ± 0.01		2
	'Colossus'	Holden 98-173-99	4.43 ± 0.06		2
	'Colossus'	Holden 2005-337	4.59 ± 0.03		2
	'Colossus'	Holden 2005-336	4.58 ± 0.03		2
	'Colossus'	Holden 2001-223A	4.56 ± 0.06		2
	'Colossus'	Holden 89-518 A	4.56 ± 0.01		2
	'Colossus'	McCracken	4.35 ± 0.12		2
	‘Halifax Hardy’ seedling	SCC 2008-100	4.56 ± 0.00		2
	ssp. *sinensis*	SCC 2008-102	4.47 ± 0.01		2
Section Manglietia					
aromatica		MGA in nursery	5.15 ± 0.05	2.58	2

changhungtana (pachyphylla)		MGA 300	4.69 ± 0.02	2.35	2
conifera var. *chingii*		MGA 378	4.67 ± 0.05	2.34	2
		Strybing	5.07 ± 0.10		2
fordiana		MGA 425	4.81 ± 0.01	2.41	2
garrettii		NCSU 2010-087	5.25 ± 0.01	2.63	2
hookeri		MGA 474	4.82 ± 0.01	2.41	2
insignis	Piroche Red Form	MGA 355	4.86 ± 0.04	2.47	2
		NCSU 2009-133	5.02 ± 0.05		2
		McCracken	4.80 ± 0.02		2
		Strybing Area 14	5.06 ± 0.01		2
kwangtungensis (moto)		MGA 435	4.65 ± 0.18	2.33	2
ovoidea		MGA in nursery	5.02 ± 0.06	2.51	2
yuyuanensis		McCracken	4.74 ± 0.01	2.37	2
		2002-041	4.73 ± 0.03		2
		MGA 160	4.73 ± 0.01		2
		Head	4.77 ± 0.02		2
Section Macrophylla					
macrophylla	White Form	Parris	4.52 ± 0.03	2.28	2
		MGA 110	4.51 ± 0.01		2
		Bartlett 2002-268	4.41 ± 0.14		2
macrophylla var. *ashei*		Parris	4.52 ± 0.03		2
macrophylla var. *dealbata*		Strybing 1986-1036	4.87 ± 0.00		2
Section Auriculata					
fraseri		SHR(wild in situ)	3.92 ± 0.04	1.94	2
		MGA (wild in situ)	3.96 ± 0.03		2
fraseri var. *pyramidata*		Bartlett 2007-0183B	3.74 ± 0.06		2
Section Kmeria					
thailandica		MGA in nursery	5.51 ± 0.02	2.76	2
Subgenus Yulania					

Section Yulania					
Subsection Yulania					
amoena		MGA 304	4.26 ± 0.12	2.13	2
biondii		MGA 027	4.12 ± 0.02	2.06	2
		Bartlett 2002-056	4.10 ± 0.04		2
campbellii		MGA 032	12.46 ± 0.09	2.09	6
		Strybing 1981-0245	12.58 ± 0.09		6
		Strybing 1997-0354	12.67 ± 0.05		6
cylindrica		MGA 216/Holden 87-86-93	8.82 ± 0.06	2.23	4
		Holden 96-111A	9.11 ± 0.11		4
		Holden 96-115B	8.99 ± 0.06		4
		Bartlett 193	8.82 ± 0.15		4
dawsoniana		Strybing 1963-0386	13.12 ± 0.10	2.19	6
denudata		Riehle 010	13.01 ± 0.05	2.21	6
		Strybing xy-0919	13.47 ± 0.03		6
kobus		Bartlett 1994-2078	4.02 ± 0.04	2.02	2
	'Ballerina'	Strybing	4.14 ± 0.03		2
	'Esveld Select'	Bartlett 2004-271	3.84 ± 0.05		2
	'Spring Snow'	NCSU	4.16 ± 0.01		2
liliiflora		Strybing xy-0972	9.34 ± 0.14	2.28	4
	'Mini Mouse'	NCSU	9.24 ± 0.03		4
	‘Nigra	Bartlett 1404	8.95 ± 0.07		4
	‘O' Neil'	NCSU 2008-258	8.95 ± 0.12		4
sargentiana		Holden 96-114	11.49 ± 0.02	1.92	6
sprengeri	'Burncoose'	Bartlett 2003-251	12.57 ± 0.19	2.11	6
	'Diva'	MGA 024	12.52 ± 0.02		6
		Strybing 1963-0368	12.93 ± 0.11		6
salicifolia		MGA 470	3.91 ± 0.02	1.96	2
	'Miss Jack'	Bartlett 2003-281	3.91 ± 0.07		2

stellata		Bartlett 1392	3.91 ± 0.02	1.97	2
	'Chysanthemumiflora'	Riehle 002	4.05 ± 0.01		2
	'Kikuzaki'	USNA 57385-H	4.12 ± 0.00		2
	'Royal Star'	Bartlett 2003-270	3.88 ± 0.03		2
	'Two Stones'	Ledvina	4.04 ± 0.05		2
zenii		MGA 440/Arnold 1545-80-B	4.12 ± 0.03	2.08	2
	Chollipo Form	SCC in nursery	4.19 ± 0.03		2
	'Pink Parchment'	Johnston	4.13 ± 0.14		2
Subsection Tulipastrum					
acuminata	'Patriot'	Ledvina	8.21 ± 0.01	2.06	4
		SCC 2010-001	8.15 ± 0.19		4
		SCC 2010-002	8.24 ± 0.01		4
		SCC 2010-003	8.14 ± 0.03		4
		SCC 2010-004	8.08 ± 0.16		4
acuminata var. *subcordata* 'Brenda'		NCSU 2004-061	8.14 ± 0.03		4
	'Skylands Best'	MGA 231	8.32 ± 0.05		4
	'Steven's Creek'	MGA 152	8.26 ± 0.05		4
Section Michelia					
cavaleriei var. *platypetala*		Strybing area 14	4.40 ± 0.08	2.19	
		Bartlett 2007-0372A	4.36 ± 0.01		
champaca		Strybing area 14	4.76 ± 0.01	2.37	2
	Orange Form	Stowe Conservatory	4.72 ± 0.06		2
chapensis		Strybing 99-0128	4.92 ± 0.02	2.46	2
doltsopa		MGA 406	4.44 ± 0.10	2.26	2
		Strybing	4.61 ± 0.01		2
ernestii		MGA 211	4.50 ± 0.03	2.25	2
figo		SCBG	4.82 ± 0.01	2.29	2
		MGA 397	4.52 ± 0.02		2
	'Port Wine'	NCSU 2009-045	4.66 ± 0.01		2

	‘Port Wine’	Bartlett 2006-0124	4.30 ± 0.03		2
	var. *skinneriana*	Parris	4.48 ± 0.08		2
	var. *crassipes*	SCC in nursery	4.71 ± 0.06		2
floribunda		MGA in nursery	4.51 ± 0.02	2.26	2
foveolata	Shibamichi Form	MGA 356	4.23 ± 0.07	2.16	2
	var. *cinerascens*	MGA 426 TH2285	4.42 ± 0.06		2
fulva var. *calcicola*		MGA in nursery	4.61 ± 0.13	2.31	2
laevifolia		MGA 424	4.63 ± 0.02	2.28	2
	‘Bubbles’	McCracken	4.52 ± 0.01		2
	‘Copperstop’	NCSU 2008-296	4.58 ± 0.03		2
	‘Gail's Favorite’	NCSU 2008-268	4.45 ± 0.07		2
	Heronswood Selection	MGA 432	4.64 ± 0.02		2
	‘Willlowleaf’	McCracken	4.42 ± 0.07		2
lanuginosa		MGA 454	4.80 ± 0.07	2.40	2
maudiae		Head	4.41 ± 0.03	2.28	2
		NCSU 2009-092	4.45 ± 0.03		2
	Yuyuan Form	MGA 188	4.87 ± 0.05		2
martinii		MGA in nursery	4.75 ± 0.04	2.38	2
odora		MGA 472	4.54 ± 0.01	2.27	2
shiluensis		MGA 385	4.49 ± 0.02	2.25	2
sirindhorniae		MGA in nursery	4.53 ± 0.16	2.27	2
Subgenus Gynopodium					
Section Gynopodium					
lotungensis	Small Leaf Form	MGA 380	11.44 ± 0.06	1.93	6
	Small Leaf Form	MGA 260	11.72 ± 0.17		6
	Large Leaf Form	MGA 367	11.57 ± 0.09		6
yunnanensis	Vietnam origin	MGA (07-SM-051)	12.50 ± 0.00	2.08	6
Section Manglietiastrum					

sinica		MGA in nursery	4.21 ± 0.02	2.11	2
Genus Liriodendron					
chinensis		Strybing Area 4A	3.47 ± 0.09	1.74	2
tulipifera	'Arnold'	NCSU 1999-292	3.35 ± 0.02	1.68	2

[z] Sources – MGA = Magnolian Grove Arboretum (R. Figlar), Pickens, SC ; NCSU = N. C. State University Mountain Horticultural Crops Research and Extension Center, Mills River, NC ; McCracken = P. McCracken, Zebulon, NC ; Strybing = Strybing Arboretum, San Francisco, CA ; Bartlett = Bartlett Tree Research Facility, Charlotte, NC; SCC = Spartanburg Community College Arboretum, Spartanburg SC ; USNA = U. S. National Arboretum, Washington, DC ; Head = R. Head, Seneca, SC ; Parris = J.K. Parris' Residential Garden, Spartanburg, SC ; Ledvina = D. Ledvina, Green Bay, WI ; Holden = Holden Arboretum, Kirtland, OH; Gilbert's = Gilbert's Nursery, Chesnee, SC; SHR = Southern Highlands Reserve, Lake Toxaway, NC; Milliken = Milliken Arboretum, Spartanburg, SC ; Riehle = R. Riehle Garden, Spartanburg, SC ; SCBG = South Carolina Botanical Garden, Clemson, SC ; KP = J.K. Parris' plants in greenhouse, Spartanburg, SC ; Johnston = J. Johnston, Clayton, GA.

[y] Genome sizes were determined using 4',6-diamidino-2-phenylindole as the flourochrome stain. Values are means ± SE.

[x] $1C_X$ values were calculated as: [(2C value / ploidy level)

Table 2.3. Relative genome size (pg) and estimated ploidy level for interspecific hybrids of *Magnolia* arranged by reported parentage ploidy levels.

Reported parentage	Cultivar/ selection	Source/ accession no.[z]	Relative 2C Genome size (pg)[y] [mean {plus minus} SE (pg)][y]	Weighted $1C_X$ Genome size (pg)[x]	Report-ed Parent-al Ploidy levels (x)[w]	Estim-ated ploidy level (x)[v]
Subgenus Magnolia						
Intraploid Hybrids						
2n=2x=38						
insignis × *virginiana*	'Katie-O'	NCSU 2004-012	4.33 ± 0.04	2.17	2×2	2
insignis × *virginiana*	'Katie-O'	MGA 307	4.27 ± 0.04	2.17	2×2	2
macrophylla × *tripetala*		MGA in nursery	3.68 ± 0.01	2.12	2×2	2
obovata × *virginiana*	'Nimbus'	NCSU 2003-041	3.79 ± 0.04	1.93	2×2	2
officinalis × *tripetala*		MGA 457	3.96 ± 0.01	1.95	2×2	2
sieboldii 'Colossus' × *insignis*		MGA in nursery	4.60 ± 0.03	2.37	2×2	2
sieboldii 'Colossus' × *insignis*		McCracken	4.63 ± 0.06	2.37	2×2	2
sieboldii 'Genesis' × *virginiana*	R10-24	Riehle 009	4.06 ± 0.01	2.06	2×2	2
sieboldii 'Genesis' × *virginiana*	R10-24	Bartlett 2007-0045A	3.93 ± 0.13	2.06	2×2	2
sieboldii 'Genesis' × *virginiana* ×*thompsoniana* (=*virginiana* × *tripetala*)	R10-24	McCracken	4.10 ± 0.01	2.06	2×2	2
		Strybing 1963-0522	3.95 ± 0.02	1.93	2×2	2
×*thompsoniana* (=*virginiana* × *tripetala*)	'Cairn Croft'	Bartlett 2007-0019	3.67 ± 0.02	1.93	2×2	2

((*tripetala* × *obovata*) × *tripetala*)) 'Silk Road' × *insignis*		MGA in nursery	4.35 ± 0.04	2.23	2×2	2
yuyuanensis × *insignis*		McCracken	4.53 ± 0.07	2.43	2×2	2
yuyuanensis × *sieboldii*	104/1	McCracken	4.67 ± 0.11	2.32	2×2	2
yuyuanensis × *virginiana*		NCSU 2009-131	4.41 ± 0.04	2.12	2×2	2
virginiana 'Havener' × *insignis* (Red Form)	111/7	McCracken	4.23 ± 0.00	2.17	2×2	2
Interploid Hybrids						
2n=3x=57						
(*grandiflora* × *virginiana*) × *virginiana*		USNA 2	5.62 ± 0.00	1.87	4×2	3
2n=4x=76						
grandiflora × *virginiana*	'Maryland'	MGA 077	7.52 ± 0.03	1.87	6×2	4
grandiflora × *virginiana*	'Maryland'	McCracken	7.45 ± 0.04	1.87	6×2	4
grandiflora × *virginiana*	'Monland'	SCBG	11.29 ± 0.07	1.87	6×2	6
insignis × *grandiflora* 'Kay Parris'		KP 2009-005	8.53 ± 0.11	2.02	2×6	4
insignis × *grandiflora* 'Kay Parris'		NCSU 2010-026-001	8.50 ± 0.09	2.02	2×6	4
sieboldii 'Colossus' × *grandiflora* 'Bracken's Brown Beauty'		McCracken	7.87 ± 0.01	1.97	2×6	4
sieboldii 'Colossus' × *grandiflora* 'Kay Parris'		KP 2008-001	8.23 ± 0.02	1.97	2×6	4
sieboldii 'Colossus' × 'Sweet Summer'		MGA 280	8.02 ± 0.10	1.97	2×6	4
sieboldii 'Pride of Norway' ×		MGA 417	7.99 ± 0.04	1.97	2×6	4

'Sweet Summer'						
virginiana var. australis ×						
grandiflora 'Samuel Sommer'	'Sweet Summer'	MGA 327	11.51 ± 0.04	1.87	2×6	6
virginiana var. australis ×						
grandiflora 'Samuel Sommer'	'Sweet Summer'	McCracken	11.54 ± 0.13	1.87	2×6	6
virginiana ×*grandiflora*		NCSU 2001-233	11.58 ± 0.09	1.87	2×6	6
2n=5x=95						
(*grandiflora* × *virginiana*)	(Maryland					
'Maryland' × *grandiflora*	Seedling)	MGA 325	9.00 ± 0.01	1.87	4×6	5
Subgenus Yulania						
Intraploid Hybrids						
2n=2x=38						
×*kewensis* (=*kobus* ×	'Wada's					
salicifolia)	Memory'	NCSU/MHCREC	4.05 ± 0.04	1.99	2×2	2
	'Wada's					
	Memory'	Bartlett 2007-0131	3.83 ± 0.02	1.99	2×2	2
×*loebneri* (=*kobus* ×						
stellata)	‘Donna’	Bartlett 2007-0281B	5.86 ± 0.04	2.00	2×2	3
	'Pink Superstar'	MGA 076	4.02 ±0.01	2.00	2×2	2
	'Leonard					
	Messel'	NCSU 1998-348	4.40 ± 0.12	2.00	2×2	2
	'Leonard	Milliken 6-0043-01-				
	Messel'	89-003	4.00 ± 0.08	2.00	2×2	2
	'Mag's					
	Pirouette'	Bartlett	3.97 ± 0.04	2.00	2×2	2
	'Merril'	MGA 085	3.86 ± 0.01	2.00	2×2	2
	'Spring Snow’	Bartlett 2004-0126A	3.86 ± 0.00	2.00	2×2	2
	'Wildcat'	MGA 248	3.71 ± 0.22	2.00	2×2	2

		Bartlett 1406	3.98 ± 0.03	2.00	2×2	2
×*alba* (=*champaca* ×						
montana)		Stowe Conservatory	4.81 ± 0.02	2.28	2×2	2
	(Clifford					
laevifolia × *figo*	Parks)	MGA 456	4.46 ± 0.04	2.28	2×2	2
×*foggii* (=*figo* × *doltsopa*)		MGA 144	4.53 ± 0.02	2.27	2×2	2
2n=4x=76						
acuminata 'Busey' ×						
acuminata sub. 'Miss						
Honeybee'	'Miranja'	Bartlett 2004-313	18.25 ± 0.52	2.10	4×4	~8.6
liliiflora 'O'Neill' × *kobus*						
'Norman Gould'	'Roseanne'	Ledvina	8.53 ± 0.08	2.15	4×4	4
×*brooklynensis* 'Woodsman'						
× (*acuminata* 'Miss						
Honeybee' × *stellata*) 'Gold						
Star'	'Solar Flair'	NCSU 2001-239	8.19 ± 0.06	2.13	4×4	4
×*brooklynensis* 'Woodsman'						
× (*acuminata* 'Miss						
Honeybee' × *stellata*) 'Gold						
Star'	'Sunburst'	NCSU 2000-065	8.07 ± 0.02	2.13	4×4	4
xbrooklynensis 'Woodsman'						
× (*acuminata* 'Miss						
Honeybee' *x stellata*) 'Gold						
Star'	'Tranquility'	Bartlett 2004-308-A	8.15 ± 0.01	2.13	4×4	4
×*brooklynensis* (=*acuminata*						
× *liliiflora*)	'Woodsman'	SCBG	8.21 ± 0.05	2.17	4×4	4
2n=6x=114						

denudata × *sprengeri* 'Diva'	'Legacy'	NCSU 1998-260	13.11 ± 0.16	2.16	6×6	6
sargentii var. *robusta* × *campbellii*	'Hawk'	Bartlett 2007-0288A	12.67 ± 0.25	2.01	6×6	6
×*veitchii* (=*campbellii* × *denudata*)		Strybing 1963-0387	12.96 ± 0.04	2.15	6×6	6
Interploid Hybrids						
cylindrica × ×*veitchii* 'Peter Veitch'	'Albatross'	MGA 004	11.14 ± 0.05	2.18	4×6	5
×*soulangeana* (=*denudata* × *liliiflora*)	'Alexandrina'	Bartlett	10.70 ± 0.06	2.24	6×4	5
×*soulangeana* (=*denudata* × *liliiflora*)	'Andre Leroy'	Milliken	14.60 ± 0.30	2.24	? ×?	~6.5
cylindrica × *denudata* 'Sawada's Pink'	'Angelica'	Bartlett 2007-0287A	10.83 ± 0.21	2.22	4×6	5
stellata × *liliiflora* 'Nigra'	'Ann'	NCSU 2006-163	6.28 ± 0.01	2.18	2×4	3
liliiflora × *cambellii* 'Lanarth'	'Apollo'	Bartlett 2007-0287A	11.02 ± 0.14	2.17	4×6	5
(*campbellii* 'Lanarth' × *liliiflora*) 'Vulcan' × ×*soulangeana* 'Lennei'	ArborTree Select	NCSU 2000-119-001	16.97 ± 0.17	2.21	5×~8	~7.7
×*soulangeana* 'Lennei Alba' × (*campbellii* 'Lanarth' × *sargentiana*) 'Mark Jury'	'Athene'	Bartlett	14.96 ± 0.19	2.14	~7.6×6	~7
×*soulangeana* 'Lennei' × (*campbellii* 'Lanarth' × *sargentiana*) 'Mark Jury'	'Atlas'	MGA 156	12.82 ± 0.18	2.14	~8×6	~6
stellata 'Rosea' × *liliiflora* 'Nigra'	'Betty'	NCSU 2006-164	6.61 ± 0.04	2.18	2×4	3
(*acuminata* × ×*brooklynensis*	'Blushing	Bartlett 2007-	10.32 ± 0.15	2.11	4×6	5

'Evamaria') 'Yellow Bird' ×	Belle'	0280B				
(sargentiana × *sprengeri*						
'Diva') 'Caerhays Belle'						
acuminata × *denudata*						
'Sawada's Cream'	'Butterflies'	NCSU 1998-259	10.71 ± 0.01	2.15	4×6	5
'Legend' × 'Butterflies'	'Coral Lake'	Riehle 008	12.09 ± 0.02	2.15	5×5	~5.6
×*veitchii* × ×*soulangeana*	'David Clulow'	Bartlett 2004-267	16.75 ± 0.31	2.19	6×?	~7.6
×*brooklynensis* 'Woodsman' ×						
(×*soulangeana* 'Lennei Alba' ×						
×*veitchii*) 'Tina Durio'	'Daybreak'	MGA 157	10.71 ± 0.01	2.20	4×~6.9	~4.9
acuminata × *denudata*	'Elizabeth'	NCSU 1998-272	10.59 ± 0.03	2.15	4×6	5
denudata × *stellata* 'Waterlily'	'Emma Cook'	MGA 197	10.26 ± 0.04	2.15	6×2	~4.8
kobus 'Norman Gould' ×						
×*soulangeana* 'Lennei'	'Eskimo'	NCSU 2000-071	9.99 ± 0.04	2.14	4×~8	~4.6
sprengeri 'Diva' × 'Wada's						
Picture'	'Felicity'	Bartlett	10.75 ± 0.10	2.18	6×5	~4.9
×*soulangeana* 'Deep Purple	'Frank's					
Dream' × ×*veitchii* 'Paul Cook'	Masterpiece'	NCSU 2001-237	14.66 ± 0.14	2.19	?×6	~6.7
×*soulangeana* (=*denudata* ×						
liliiflora)	'Fukuju'	Bartlett	19.02 ± 0.06	2.24	?×?	~8.5
liliiflora 'Nigra' × *sprengeri*						
'Diva'	'Galaxy'	Bartlett 2002-724	10.45 ± 0.11	2.18	4×6	5
acuminata var. *sub.* 'Miss						
Honeybee' × *denudata*						
'Sawada's Cream'	'Gold Finch'	NCSU 2000-261	10.81 ± 0.13	2.15	4×6	5.0
		Bartlett 2007-				
acuminata × *denudata*	'Golden Sun'	0365A	13.59 ± 0.12	2.15	4×6	~6.3
acuminata × *stellata*	'Gold Star'	NCSU 2004-063	8.22 ± 0.06	2.06	4×2	4

×*soulangeana* (=*denudata* × *liliiflora*)	'Grace McDade'	Bartlett 2004-238	17.35 ± 0.14	2.24	?×?	~7.8
(×*brooklynensis* 'Woodsman' × ×*soulangeana* 'Lennei') × (*acuminata* × *denudata*) 'Elizabeth'	'Green Snow'	Bartlett 2004-236	11.47 ± 0.15	2.20	(4×~8)×5	~5.2
×*brooklynensis* 'Woodsman' × (*acuminata* × *denudata*) 'Elizabeth'	'Hot Flash'	Bartlett 2007-0367A	8.43 ± 0.07	2.15	4×5	~3.9
(*campbellii* 'Lanarth' × *sargentiana*) 'Mark Jury' × ×*soulangeana* 'Lennei'	'Iolanthe'	MGA 407	13.62 ±0.05	2.14	6×~8	~6.4
acuminata × *denudata*	'Ivory Chalice'	NCSU 1998-262	10.76 ± 0.07	2.17	4×6	5
×*soulangeana* × ×*veitchii*	'Jon Jon'	NCSU 2000-258	15.16 ± 0.08	2.19	?×6	~6.9
×*loebneri* 'Encore' × ×*soulangeana* 'Alexandrina'	Kehr Seedling	NCSU 2001-143-001	10.92 ± 0.09	2.17	2×~5	~5
acuminata × *denudata*	'Legend'	NCSU 1998-261	10.77 ± 0.02	2.15	4×6	5
×*soulangeana* (=*denudata* × *liliiflora*)	'Lennei'	Bartlett 1075	17.89 ± 0.16	2.24	?×?	~8
×*soulangeana* (=*denudata* × *liliiflora*)	'Lennei Alba'	Bartlett 1995-2153	16.91 ± 0.12	2.24	?×?	~7.6
acuminata var. *subcordata* × ×*soulangeana* 'Big Pink'	'Limelight'	Bartlett 2007-0495C	14.23 ± 0.13	2.18	4×?	~6.5
acuminata × (*acuminata* × *denudata*)	'Lois'	Riehle 001	14.61 ± 0.28	2.10	4×5	~7.0
×*soulangeana* × ×*veitchii*	'Manchu Fan'	Bartlett 2003-593	14.86 ± 0.04	2.19	?×6	~6.8
(*liliiflora* × *cylindrica*) ×	'March till	NCSU 2001-257	12.89 ± 0.14	2.25	4×?	~5.7

×*soulangeana* 'Ruby'	Frost'					
×*soulangeana* 'Lennei' ×						
(×*soulangeana* 'Lennei'						
seedling × *sprengeri 'Diva'*)		Bartlett 2007-				
'Paul Cook'	'Millie Gaylon'	0496A	14.20 ± 0.16	2.21	~8×(?×6)	~6.4
stellata × *liliiflora*	'Orchid'	Bartlett 2002-430	6.44 ± 0.24	2.18	2×4	3
acuminata × ×*veitchii* 'Peter						
Veitch'	'Pastel Beauty'	NCSU 2000-240	10.12 ± 0.06	2.13	4×6	5
acuminata × *sprengeri* 'Diva'	'Peachy'	Bartlett 2003-286	10.11 ± 0.11	2.11	4×6	5
×*soulangeana* (=*denudata* ×	'Pickard's					
liliiflora)	Firefly'	Bartlett 2004-250	17.06 ± 0.39	2.24	?×?	~7.6
liliiflora 'Reflorescens' ×						
stellata 'Rubra'	'Pinkie'	Bartlett 2003-714	6.47 ± 0.02	2.18	4×2	3
liliiflora × ×*soulangeana*	'Purple Prince	Bartlett 2003-285	10.91 ± 0.15	2.26	4×?	~4.8
liliiflora 'Nigra' × *stellata*	'Randy'	Bartlett 2004-643	6.44 ± 0.04	2.18	4×2	3
acuminata × (×*soulangeana*						
'Wada's Picture' × *sprengeri*						
'Diva') 'Big Dude'	'Red Baron'	Bartlett 2004-311	13.19 ± 0.23	2.14	4×(?×6)	~6.2
liliiflora × ×*veitchii*	'Royal Crown'	Bartlett 2002-157	10.58 ± 0.01	2.20	4×6	5
liliiflora × ×*veitchii*	'Sayonara'	NCSU 2008-266	14.82 ± 0.10	2.20	4×6	~6.7
liliiflora × (*campbellii*						
'Lanarth' × *sargentiana*) 'Mark						
Jury'	'Serene'	Bartlett 2003-263	10.59 ± 0.06	2.12	4×6	5
denudata × (*campbellii*						
'Lanarth' × *liliiflora)* 'Vulcan'	'Shiraz'	Bartlett 2003-277	12.76 ± 0.16	2.19	6×5	~5.8
liliiflora 'Nigra' *x sprengeri*						
'Diva'	'Spectrum'	MGA	11.58 ± 0.02	2.18	4×6	5
campbellii × *liliiflora*	'Star Wars'	MGA 330	10.53 ± 0.08	2.17	6×4	5
acuminata × *denudata*	'Sunray'	Bartlett 2007-	10.22 ± 0.17	2.17	4×6	5

		0358A				
×*brooklynensis* 'Woodsman' × (*acuminata* × *denudata*) 'Elizabeth'	'Sunsation'	SCC nursery	14.73 ± 0.33	2.18	4×5	~6.8
liliiflora × *stellata* 'Rosea'	'Susan'	Bartlett 2002-433	6.58 ± 0.01	2.18	4×2	3
×*soulangeana* 'Lennei Alba' × ×*veitchii*	'Tina Durio'	MGA 087	15.23 ± 0.02	2.20	~7.6×6	~6.9
×*veitchii* × ×*soulangeana* 'Rustica Rubra'	'Todd Gresham'	Bartlett 2002-641	14.75 ± 0.33	2.19	6×?	~6.7
campbellii 'Lanarth' × *liliiflora* hybrid	'Vulcan'	Riehle 004	10.54 ± 0.11	2.17	6×4	5
acuminata var. subcordata × ×*soulangeana* 'Alexandrina'	'Yellow Lantern'	Bartlett 2003-266	14.43 ± 0.26	2.18	4×5	~6.6
acuminata × *denudata* (*cylindrica* × *denudata*) 'Pegasus' × *campbellii* 'Darjeeling'	'Yellow Sea' 'Zeal'	Bartlett 2004-0495C Bartlett 2005-0025	8.68 ± 0.01 10.15 ± 0.26	2.17 2.17	4×6 5×6	4 ~4.6
acuminata × *figo* (*liliiflora* 'Nigra' × *sprengeri* 'Diva') 'Galaxy' × *campbellii* var. *mollicomata*		MGA 120 MGA 153	6.16 ± 0.06 12.34 ± 0.01	2.16 2.13	4×2 5×6	3 ~5.8
×*veitchii* 'Isca' × *liliiflora*		MGA 109	10.84 ± 0.01	2.13	6×4	5
cylindrica hybrid (Polly Hill)		MGA 215	13.35 ± 0.04	2.23	4×?	6.0
cylindrica hybrid		SCC 2009-004	14.92 ± 0.27	2.23	8×?	~6.7
cylindrica hybrid		SCC 2009-005	15.21 ± 0.11	2.23	8×?	~6.8

[z] Sources – MGA = Magnolian Grove Arboretum (R. Figlar), Pickens, SC ; NCSU = N. C. State University Mountain Horticultural Crops Research and Extension Center, Mills River, NC ; McCracken –

P. McCracken, Zebulon, NC ; Strybing – Strybing Arboretum, San Francisco, CA ; Bartlett – Bartlett Tree Research Facility, Charlotte, NC ; SCC – Spartanburg Community College Arboretum, Spartanburg, SC ; USNA – U.S. National Arboretum, Washington, DC ; Head – R. Head, Seneca, SC ; Parris – J.K. Parris' Residential Garden, Spartanburg SC ; Ledvina = D. Ledvina, Green Bay, WI ; Holden = Holden Arboretum, Kirtland, OH; Gilbert's = Gilbert's Nursery, Chesnee, SC ; SHR = Southern Highlands Reserve, Lake Toxaway, NC; Milliken = Milliken Arboretum, Spartanburg, SC ; Riehle = R. Riehle Garden, Spartanburg, SC ; SCBG = South Carolina Botanical Garden, Clemson, SC ; KP = J.K. Parris' plants in greenhouse, Spartanburg, SC

[y] Genome sizes were determined using 4',6-diamidino-2-phenylindole as the flourochrome stain. Values are means ± SE.

[x] Weighted $1C_X$ values were calculated as: [($1C_X$ value of the female parent × ploidy level of the female parent/2) + ($1C_X$ value of the male parent × ploidy level of the male parent/2)]/[(ploidy level of the female parent + ploidy level of the male parent)/2] . When the $1C_X$ was not known for the exact parent, then an average for the parental species or section was used.

[w] Parental ploidy and genome sizes for *M.* ×*soulangeana* hybrids are unknown and marked as "?".

[v] Estimated ploidy levels were calculated as: 2C genome size / weighted $1C_X$ value. If both parent species had even ploidy levels, then ploidy levels of the progeny were rounded to the nearest whole numbers if supported by an appropriate relative genome size. If either parent had an odd ploidy level, then ploidy levels of the progeny were rounded to the nearest 0.01 to reflect apparent aneuploidy.

Table 2.4. Relative genome sizes (pg) and estimated ploidy levels of artificially induced polyploid *Magnolia* spp.

Taxa	Source/Accession no.[z]	Relative 2C Genome size (pg) [mean {plus minus} SE (pg)] [y]	Estimated Ploidy level (*x*)[x]
cylindrica	Holden 92-443A	17.49 ± 0.01	8
	Holden 92-443F	17.42 ± 0.30	8
	Holden 92-443Q	17.40 ± 0.13	8
	Holden 92-443E	17.45 ± 0.58	8
	Holden 92-443P	17.36 ± 0.11	8
	Holden 92-443L	17.27 ± 0.04	8
	Holden 92-443J	17.28 ± 0.05	8
	Holden 92-443I	17.07 ± 0.11	8
	Holden 92-443G	17.31 ± 0.09	8
grandiflora 'Little Gem' (cytochimera)	Head	11.11 ± 0.09	6
		21.80 ± 0.32	12
kobus 'Norman Gould'	USNA 59598-H	7.79 ± 0.00	4
stellata	Holden 97-103F	8.31 ± 0.17	4
	Holden 97-103M	8.10 ± 0.12	4
	Holden 97-103C	8.17 ± 0.04	4
	Holden 97-103Q	8.23 ± 0.07	4
	Holden 97-103U	8.20 ± 0.00	4

[z] Sources = Holden = Holden Arboretum, Kirtland, OH; Head = R. Head, Seneca, SC ; USNA = U. S. National Arboretum, Washington, DC.

[y]Genome sizes were determined using 4',6-diamidino-2-phenylindole as the flourochrome stain. Values are means ± SE.

[x] Estimated ploidy levels were calculated as: 2C genome size / $1C_X$ value (2.23 for *M. cylindrica*, 1.87 for *M. grandiflora*, 2.02 for *M. kobus*, and 1.97 pg for *M. stellata*) and rounded to the closest whole number.

Table 2.5. Comparison of differential staining of fluorochromes and DNA base pair content for selected species from 10 sections of *Magnolia*.

Taxa	Source/ Accession no.[z]	Genome size (pg)[y] DAPI	PI	Difference[x]	AT%[w]
Subgenus Magnolia					
Section Gwillimia, *M. delavayii*	MGA 411	5.13	5.86	0.73 *	63.91 A
Section Auriculata, *M. fraseri*	MGA wild in situ	3.85	4.01	0.16 *	63.23 B
Section Macrophylla, *M. macrophylla*	Parris 1996-001	4.54	4.79	0.25 *	62.46 B
Section Magnolia, *M. virginiana* 'Jim Wilson'	NCSU 2004-204	3.73	4.00	0.27 *	62.68 B
Section Rhytidospermum, *M. rostrata*	NCSU 2008-028	4.51	4.67	0.16 *	62.09 CD
Section Manglietia, *M. yuyuanensis*	NCSU 2002-041	4.77	4.90	0.13[NS]	61.97 CD
Subgenus Yulania					
Section Yulania, *M. stellata* 'Royal Star'	NCSU 2008-157	3.93	4.04	0.11[NS]	61.97 CD
Section Michelia, *M. laevifolia* 'Michelle'	NCSU 2008-244	4.35	4.55	0.20 *	62.29 BC
Subgenus Gynopodium					
Section Gynopodium, *M. lotungensis*	Parris, 1997-001	12.27	12.94	0.67 *	62.44 BC
Section Manglietiastrum, *M. sinica*	MGA, 2007a	4.21	4.24	0.03[NS]	61.60 D

[z] Sources – MGA = Magnolian Grove Arboretum (R. Figlar), Pickens, SC ; NCSU = N. C. State University Mountain Horticultural Crops Research and Extension Center, Mills River, NC ; Parris – J.K. Parris' Residential Garden, Spartanburg, SC

[y] Genome size, n=5, determined using either 4',6-diamidino-2-phenylindole (DAPI) or propidium iodide (PI).

[x] Difference between PI and DAPI methods. Fisher's Least Significant Difference ($LSD_{0.05}$) (comparing DAPI to PI for a given taxa within a row) = 0.13; *, significant; NS, not significant.

[w] % AT composition. Mean separation within column (among taxa) by $LSD_{0.05}$ = 0.56

Figure 2.1

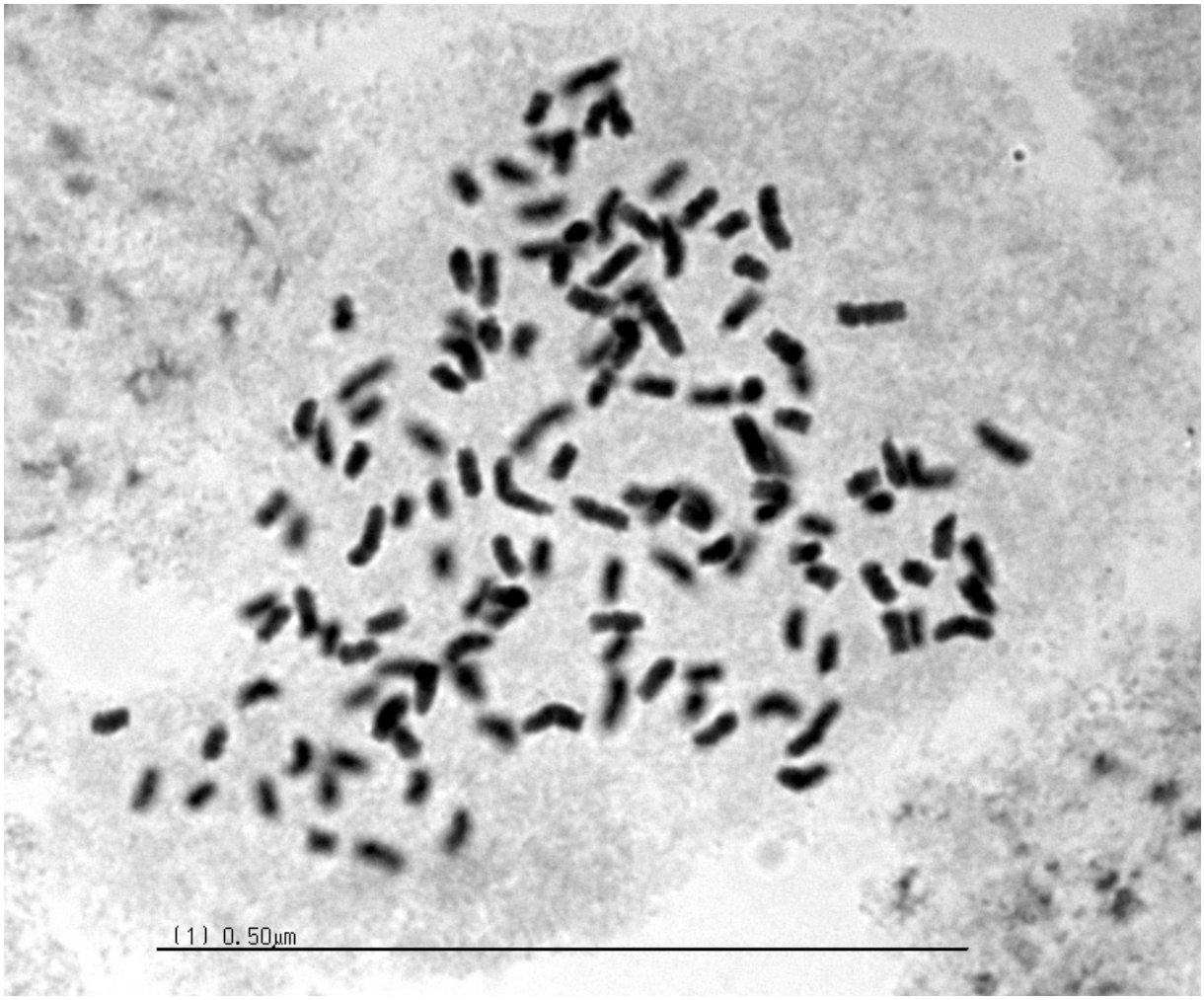

Figure 2.1 Photomicrograph of a root tip cell of *Magnolia* SCC 2009-004 in early metaphase, with approximately 133 chromosomes. Maternal parent *Magnolia cylindrica* (2*n*=8*x*=152), paternal parent unknown, but likely (2*n*=6*x*=114), resulting in a plant that is 7*x*.

CHAPTER THREE

SELECTING BASAL SALT COMPOSITION, CYTOKININ, AND PHENOLIC BINDING AGENTS FOR *IN VITRO* GROWTH AND *EX VITRO* ESTABLISHMENT OF *MAGNOLIA* 'ANN'

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INTRODUCTION

The genus *Magnolia*. consists of over 250 species (Figlar and Nooteboom, 2004), and numerous hybrids and cultivars that can be cultivated in temperate and tropical climates worldwide. *Magnolia* (*liliiflora* 'Nigra'(4*x*)× *stellata* 'Rosea'(2*x*)) 'Ann' (NA 28344; PI 326570) is a member of the 'Little Girl' series of magnolias that have become widely popular (USNA 2003). *Magnolia* 'Ann' is characterized by a desirable combination of traits including prolific and remontant flowering and a shrubby form. In an extensive study of genome size among a wide range of cultivars, Parris et al. (2010) found *M.* 'Ann' to be a triploid (2n=3*x*=57) and due to triploidy is sterile. *In vitro* regeneration procedures may provide an efficient means for rapid, large-scale production as well as providing a platform for *in vitro* chromosome doubling that may restore fertility and allow for future breeding options.

Previous *in vitro* propagation studies on magnolia have focused on species for conservation purposes, including *M. acuminata* var. *cordata* (Merkle and Wiecko 1990, Merkle and Wilde 1995), *M. dealbata* (Mata-Rosas et al. 2006), *M. denudata* (Bi et al.

2002), *M. fraseri* (Merkle and Wiecko 1990, Merkle and Wilde 1995), *M. macrophylla* (Merkle and Watson-Pauley 1993, Merkle and Wilde 1995), *M. obovata* (Kim et al. 2007), *M. officinalis* (Tong et al. 2002), *M. pyramidata* (Merkle and Watson-Pauley 1994,Merkle and Wilde 1995), *M. sieboldii* (Lu et al. 2008), *M. sinicum* (JunLi and Mingdong 2007), and *M. virginiana* (Merkle and Wiecko 1990, Merkle and Wilde 1995). However, less work has been done on micropropagation of ornamental *Magnolia* taxa with the exception of *M. × soulangeana* (Maene and DeBergh 1985, Kamenicka and Lanakova 2000, Marinescu 2008), *M. grandiflora* (Sakr et al. 1999, Tan et al. 2003) and *M. delavayi* (Luo and Sung 1996). Beidermann (1987) addresses micropropagation of *M. stellata*, and the hybrids 'Elizabeth' and 'Yellow Bird'. These studies have indicated that media composition and plant growth regulators are important factors influencing the *in vitro* propagation of magnolia.

Media comprised of MS basal salts and vitamins, (Murashige and Skoog 1962) has been widely used for the *in vitro* propagation of magnolia (Beidermann 1987, Kamenicka and Lanakova 2000 Marinescu 2008). Additionally, Merckle and Watson-Pauley (1993) used Blaydes Modified Basal Medium (Blaydes 1966) for the somatic embryogenesis of *Magnolia sp*. Several alternative media compositions; Driver and Kuniyuki basal salt mixture (DKW) (Driver and Kuniyuki 1984) and Lloyd and McCown Woody Plant Medium (WPM)(Lloyd and McCown 1981) have been tested with a wide range of woody plant species but there were relatively few investigations with Magnolia.

While several cytokinins have been used to induce shoot proliferation, 6-benzylaminopurine (BAP) is most often used for *Magnolia*. For *Magnolia*

×*soulangeana*, BAP was shown to produce higher shoot regeneration than 6-(γ,γ-dimethylallylamino) purine (2iP), kinetin or thidiazuron (Marinescu 2008). However, BAP has been shown to induce hyperhydricity, reduce shoot quality, and inhibit rooting. *Meta*-topolin (*m*T), a naturally occurring cytokinin similar in structure to BAP, has not been associated with hyperhydricity (Bairu et al., 2007; Werbrouck et al., 1996), and has been effective for micropropagation of many species. (Meyer, et al, 2010, Amoo et al 2011).

Shoot proliferation of magnolias during micropropagation has been reported to be difficult due to the presence of phenolic substances (JunLi and Mingdong 2007, Sakr et al. 1999). Activated charcoal (AC) and polyvinylpyrrolidone (PVP) are commonly used in media to bind phenolics and ascorbic acid may reduce oxidative processes that lead to phenolic accumulation. Radomir and Radu (2008) incorporated ascorbic acid in the effective micropropagation of *Magnolia* ×*soulangeana*. While AC and PVP have not been evaluated for *Magnolia* they have been effective phenolic binding agents used in the micropropagation of many plant species (Roy 1991, Thomas 2008).

The objective of the current study was to evaluate a range of basal media compositions, cytokinins, and phenolic binding agents, in a series of experiments to improve *in vitro* growth conditions for *M.* 'Ann' as a platform for micropropagation and future ploidy manipulation. *Ex vitro* establishment protocols were also examined to

insure that a viable pathway for commercial introduction of improved plants can be established.

MATERIALS AND METHODS

Plant Material

Magnolia 'Ann' stock was obtained and established into cultures maintained on MS basal salts and vitamins, 2 µM BAP, 30 g/L sucrose, 0.1 g/L myo-Inositol, 0.1 g/L MES monohydrate, and solidified with 0.8% agar at the N.C. State Mountain Crop Improvement Lab in Mills River, N.C. Cultures were maintained at 23 ± 2 °C using a 16h photoperiod (PPFD 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$) supplied by cool white fluorescent light.

Media Composition

The effect of basal media composition was tested with five basal salt compositions and vitamins (MS, ½ MS, WPM, Blaydes, and DKW), in factorial combination with phenolic binding agents (control, 1 g/L AC, or 1 g/L PVP). All media were supplemented with 30 g/L sucrose, 2 µM BAP, 0.1 g/L myo-Inositol, 0.1 g/L MES monohydrate, and solidified with 0.8% agar. The experiment consisted of six replicates (jars) per treatment and five subsamples (subcultured explants) per replicate arranged in a completely randomized design. Cultures were maintained under same conditions used to establish plant material (as above). After eight weeks, data were collected on shoot number, shoot length, root number, fresh weight, and dry weight.

Cytokinin Concentration

In a second experiment, the effect on plant growth of three cytokinins; BAP, *m*T, and 2iP at three concentrations (2, 4, and 8 µM) with or without 1 g/l charcoal was evaluated in a completely randomized design with a factorial arrangement of treatments. Based on the results of the first experiment, basal media consisted of MS basal salts and vitamins, 30 g/L sucrose, 0.1 g/L myo-Inositol, and 0.1 g/L MES monohydrate solidified with 0.8% agar. The experiment consisted of six replicates (jars) per treatment and five subsamples (subcultured explants) per replicate, arranged in a completely randomized design under standard culture conditions. After eight weeks, data were collected on shoot number, shoot length, root number, root length, fresh weight, and dry weight.

Data for both studies were subjected to analysis of variance (Proc GLM, SAS version 9.1; SAS Institute, Cary, NC). Means separations were based on Fisher's least significant difference (LSD).

Root Initiation and Acclimatization

The effects of rooting were investigated in response to IBA in combination with 0 or 1g/L activated charcoal as a phenolic binding agent. Stock cultures were maintained as in the previous experiments and subcultured explants were transferred to media consisting of ½ MS basal salts and vitamins, 30 g/L sucrose, 0.1 g/L myo-Inositol, 0.1 g/L MES monohydrate, and the varying IBA concentrations (0 µM, 5 µM, 10 µM and 20 µM), solidified with 0.8% agar. The experiment consisted of six replicates (jars) per treatment and five subsamples (subcultured explants) per replicate, arranged in a completely randomized design. After six weeks explants were evaluated for the

development of roots and root length produced *in vitro.* Microcuttings were carefully rinsed to reduce transfer of sucrose to the soilless media.. Microcuttings were inserted with 1 leafless node placed below the surface of the media (pine bark: perlite 2:1 v/v) in 50 round cell propagation sheets within 1020 open flats in a randomized block design. The propagation environment was intermittent mist in an open bench setting. Data was collected on number of plants rooted, roots per plant, lateral root development, and leaf development at 6 weeks. Data were subjected to regression analysis (Proc GLM, SAS version 9.1; SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Effects of Media and Phenolic Binding Agents

Media composition, phenolic binding agents and their interaction had significant effects on shoot number, root number, fresh weight and dry weight ($P<0.05$) (Table 3.1). Shoot number was greatest (3.2) in MS media with no phenolic binding agent. In general, the number of shoots produced per explant was lower on both Blaydes media and media supplemented with AC. PVP did not significantly affect shoot number in any of the treatments, but shoot length was reduced compared to AC. Leaf color and vigor (data not presented) appeared to be superior on treatments which incorporated AC (Figure 3.2). AC may also influence nitrogen uptake. For *Lagerstoemia indica*, explants grown in media with AC were able to uptake both NO^{3-} and NH^{4+} ions while cultured on media without AC could only uptake NH^{4+} (Eymar, et. al. 2001). A significant reduction in availability of nutrient and plant growth regulators, including BAP, may influence growth and reduce

shoot proliferation and lead to increases in shoot length. Fresh weight was significantly lower on Blaydes and WPM compared to other media as well as media supplemented with activated charcoal, while phenolic binding agents (PVP and AC), as well as WPM and DKW reduced dry weight. WPM and DKW have lower NH^{4+} concentrations than MS, potentially contributing to this result. Fresh and dry weight were highest (8.1 g and 0.69g) on ½ MS with no phenolic binding agent, indicating this media better conditions explants for subsequent rooting and establishment. Root number was generally higher on WPM media and media containing charcoal, though the influence of phenolic binding agents varied by media. Both media composition and phenolic binding agents significantly influenced shoot length, although there was no interaction. Shoot length was typically less on Blaydes media and significantly longer on media containing AC (Table 3.1). These results indicate that to optimize multiplication and plant conditioning for rooting and *ex vitro* establishment, two customized subcultures will be necessary.

Response to cytokinin concentration and phenolic binding agent

In the second experiment, the cytokinin composition and concentration, in the presence and absence of AC was examined. There was a significant interaction between cytokinin and AC that influenced shoot number, shoot length, fresh weight, and dry weight; while a complex interaction between cytokinin, cytokinin concentration, and AC affected dry weight (Table 3.2). In general, shoot number was higher on media containing BAP, regardless of concentration, and lower on media containing AC. Fresh weight was lower on media containing BAP, but within the BAP treatment, higher on

media containing AC. Fresh weight typically decreased in the *m*T and 2iP treatments that contained AC. Due the complex interaction stated above, *m*T had the lowest dry weight.

Similar to our study of Magnolia 'Ann', reduced shoot proliferation and increased shoot elongation and rooting in response to charcoal have also been found for *Acacia mearnsii* and *Anacardium occidentale* (cashew) (Thomas 2008). The porous and adsorptive nature of AC produces a variety of interactions with nutrients and plant growth regulators varying the growth and development of the plant species being cultured (Thomas 2008). AC has a strong absorptive capacity towards cytokinins including BAP (Ebert et al 1993, Thomas 2008) which may explain decreases in shoot proliferation. Ebert et al (1993) showed that in media containing 2.5 g/L AC, less than 2% of BAP was available after 3 days.

In the current study, BAP (2.48× (mean for all concentrations)) was e a more active in inducing shoot proliferation than either *m*T (1.41×) or 2 iP (1.08×). Similarly Marinescu (2008) obtained higher proliferation rates using BAP compared to 2iP, Kinetin or TDZ for *M.* ×*soulangeana*. Though *m*T has been reported to produce longer, greener and less hyperhydrated shoots in *Spathiphyllum floribundum* and may be an alternative cytokinin to BAP (Werbrouck et al. 1996), there was a significant reduction in shoot numbers compared to BAP in this study. As stated in the initial study comparing media; two customized subcultures will be necessary to achieve optimal multiplication and conditioning for *ex vitro* establishment.

In vitro and ex vitro root initiation and establishment

Activated charcoal significantly enhanced root formation both *in vitro* and after plants were transferred *ex vitro*. A limited number of roots were observed *in vitro* (Figure 3.4), and regression analysis (linear and quadratic) showed IBA had no significant effect on root development. There was also no significant interaction between AC and IBA (Table 3.3). *In vitro* roots mostly occurred on plantlets treated with AC (Figure 3.3). This response has been observed in numerous genera (Thomas 2008). Interestingly, *in vitro* application of AC also significantly influenced *ex vitro* rooting (Table 3.3). Given that plants were previously subcultured on media containing 2 µM BAP, residual *in vivo* BAP or related byproducts may have inhibited the effects of IBA. The addition of AC *in vitro* may have bound this residual BAP as it did in the previous experiments. Though IBA concentration produced no significant effect on root initiation *in vitro* or *ex vitro*, 5 µM IBA gave the highest mean rooting in each phase and a significant increase in leaf production with or without AC. Increased leaf number appeared to lead to plants with greater vigor (pers. obs.). Foliage produced *ex vitro* developed the more typical acuminate leaf morphology of this cultivar with greater surface area for photosynthesis and subsequent growth (Figure 3.5).

CONCLUSION

Simple effects from various interactions make sequential incorporation of cytokinins and auxins necessary. This study demonstrated MS media supplemented with 2 µM BAP and no phenolic binding agent was best for multiplication, while ½ MS media containing 1g/L AC may be used to produce microcuttings suitable for rooting and *ex*

vitro establishment. Since the i*n vitro* presence of IBA in this transition media does not produce statistically significant benefits in root formation, a quick dip application after exposure to AC, should more efficiently promote *ex vitro* rooting and establishment. Further refinement of protocols should involve: 1) determination of the optimal timing and duration of exposure to AC in the transition media, 2) developing a more streamlined method for introduction of AC to subcultures, and 3) testing these protocols on other taxa within Genus *Magnolia*. Results observed in this study may also lead to improved protocols for future experiments focused on the development of allopolyploids to restore fertility through chromosome doubling.

Table 3.1. Growth responses to different *in vitro* culture media and phenolic binding agents.

Media	Phenolic Binding Agent	Shoot Number[1]	Shoot Length (mm)[1]	Root Number[1]	Fresh Weight (g)[1]	Dry Weight (g)[1]
MS	none	3.2±0.2^{A}	17.2±1.8^{C}	0.2±0.07^{DE}	5.6±0.60^{B}	0.56±0.04^{AB}
	PVP	2.8±0.3^{AB}	20.0±2.8^{BC}	0.1±0.10^{DE}	4.8±0.45^{B}	0.58±0.04^{AB}
	AC	1.1±0.1^{C}	24.2±1.7^{AB}	0.6±0.03^{BC}	2.8±0.22^{CD}	0.42±0.03^{BC}
½ MS	none	2.8±0.2^{AB}	24.4±2.6^{AB}	0.3±0.14^{CDE}	8.1±1.21^{A}	0.69±0.08^{A}
	PVP	2.6±0.2^{AB}	19.9±1.1^{BC}	0.1±0.04^{DE}	4.6±0.35^{BC}	0.53±0.04^{B}
	AC	1.1±0.1^{C}	22.2±1.7^{B}	0.3±0.08^{CDE}	2.1±0.24^{D}	0.35±0.03^{C}
WPM	none	2.3±0.2^{AB}	19.9±1.9^{BC}	0.7±0.20^{B}	2.4±0.38^{D}	0.39±0.06^{BC}
	PVP	2.6±0.3^{AB}	16.5±1.6^{C}	0.4±0.19^{BCD}	1.9±0.57^{D}	0.33±0.05^{D}
	AC	1.2±0.1^{C}	22.4±1.3^{B}	1.8±0.16^{A}	2.4±0.16^{D}	0.42±0.03^{BC}
DKW	none	2.6±0.4^{AB}	22.7±3.0^{B}	0.1±0.04^{DE}	4.9±1.07^{B}	0.50±0.04^{B}
	PVP	2.9±0.4^{A}	19.0±1.0^{BC}	0.0±0.00^{E}	4.2±0.79^{BC}	0.49±0.08^{BC}
	AC	1.1±0.1^{C}	30.1±4.1^{A}	0.7±0.20^{BC}	3.0±0.53^{C}	0.29±0.04^{D}
Blaydes	none	1.2±0.2^{C}	8.2±2.1^{D}	0.3±0.12^{CDE}	2.2±0.24^{D}	0.62±0.09^{AB}
	PVP	1.5±0.2^{C}	13.6±3.2^{CD}	0.1±0.11^{DE}	2.2±0.44^{D}	0.45±0.06^{BC}
	AC	0.9±0.1^{C}	14.2±1.3^{CD}	0.6±0.10^{BC}	0.9±0.16^{D}	0.47±0.04^{BC}
Analysis of Variance[2]						
Media		**	**	**	**	**
PBA		**	**	**	**	**
Media x PBA		*	NS	**	**	*

[1]Values represent means ± SEM. Means followed by different letters within columns are significantly different, P<0.05.
[2]NS, *, **: Nonsignificant or significant at p=0.05 and 0.01, respectively.
PBA=Phenolic Binding Agent, AC=Activated Charcoal

Table 3.2. Growth responses to different concentrations of cytokinins and phenolic binding agents *in vitro*.

Cytokinin	Conc. (µM)	Phenolic Binding Agent	Shoot Number[1]	Shoot Length (mm)[1]	Root Number[1]	Fresh Weight (g)[1]	Dry Weight (g)[1]
BAP	2	None	2.64±0.4 ^{A}	18.6±1.9 ^{ABC}	0.00^{B}	1.00±0.0 ^{D}	0.23±0.03^{A}
		AC	1.00±0.0 ^{C}	18.2±1.0 ^{ABC}	1.40±0.5^{A}	1.33±0.1 ^{D}	0.18±0.01^{AB}
	4	None	2.40±0.1 ^{A}	19.2±0.8 ^{ABC}	0.00^{B}	1.00±0.0 ^{D}	0.20±0.02^{A}
		AC	1.08±0.1 ^{C}	17.0±0.7 ^{BCD}	0.60±0.2^{AB}	1.34±0.2 ^{D}	0.17±0.02^{B}
	8	None	2.40±0.2 ^{A}	22.5±0.7 ^{A}	0.00^{B}	1.00±0.0 ^{D}	0.19±0.02^{A}
		AC	1.15±0.1 ^{BC}	18.9±1.7 ^{ABC}	1.00±0.6^{A}	1.37±0.2 ^{CD}	0.14±0.01^{BC}
mT	2	None	1.40±0.2 ^{BC}	13.6±1.7 ^{D}	0.00^{B}	2.00±0.0 ^{BC}	0.11±0.01^{C}
		AC	1.25±0.2 ^{BC}	19.7±2.2 ^{AB}	0.75±0.5^{AB}	1.99±0.3 ^{BC}	0.20±0.02^{A}
	4	None	1.33±0.2 ^{BC}	15.4±1.8 ^{CD}	0.00^{B}	2.00±0.0 ^{BC}	0.16±0.03^{BC}
		AC	1.06±0.1 ^{C}	16.7±3.0 ^{BCD}	0.33±0.3^{B}	1.65±0.4 ^{BC}	0.13±0.01^{BC}
	8	None	1.55±0.3 ^{B}	14.9±1.4 ^{CD}	0.00^{B}	2.00±0.0 ^{BC}	0.14±0.03^{BC}
		AC	1.13±0.1 ^{BC}	15.5±0.1 ^{CD}	0.00^{B}	2.33±0.3 ^{AB}	0.13±0.01^{BC}
2iP	2	None	1.06±0.1 ^{C}	19.6±2.5 ^{ABC}	0.00^{B}	3.00±0.0 ^{A}	0.21±0.04^{A}
		AC	1.10±0.1 ^{BC}	17.9±2.8 ^{ABCD}	1.00±0.4^{A}	1.84±0.4 ^{BC}	0.18±0.02^{AB}
	4	None	1.12±0.1 ^{BC}	13.9±1.7 ^{D}	0.00^{B}	3.00±0.0 ^{A}	0.13±0.01^{BC}
		AC	1.20±0.1 ^{BC}	19.0±0.7 ^{ABC}	0.83±0.2^{AB}	2.01±0.2 ^{B}	0.22±0.02^{A}
	8	None	1.06±0.1 ^{C}	20.7±1.8 ^{AB}	0.67±0.7^{AB}	2.37±0.6 ^{AB}	0.13±0.02^{BC}
		AC	1.00±0.0 ^{C}	17.9±1.2 ^{ABCD}	0.00^{B}	3.00±0.0 ^{A}	0.23±0.02^{A}
Analysis of Variance[2]							
Cytokinin			**	*	NS	**	**
Conc.			NS	NS	NS	NS	NS
AC			**	NS	**	NS	NS
Cytokinin x Conc.			NS	NS	NS	NS	NS
Cytokinin x AC			**	*	NS	**	**

Conc. x AC	NS	NS	NS	*	NS
Cytokinin x Conc. x AC	NS	NS	NS	NS	**

[1]Values represent means ± SEM. Means followed by different letters within columns are significantly different, P<0.05.
[2]NS, *, **: Nonsignificant or significant at p=0.05 and 0.01, respectively. Conc. = Concentration;; AC= 1g/L Activated Charcoal.

Table 3.3. *In vitro* and *ex vitro* growth responses to different *in vitro* IBA concentrations and phenolic binding agents (PBA).

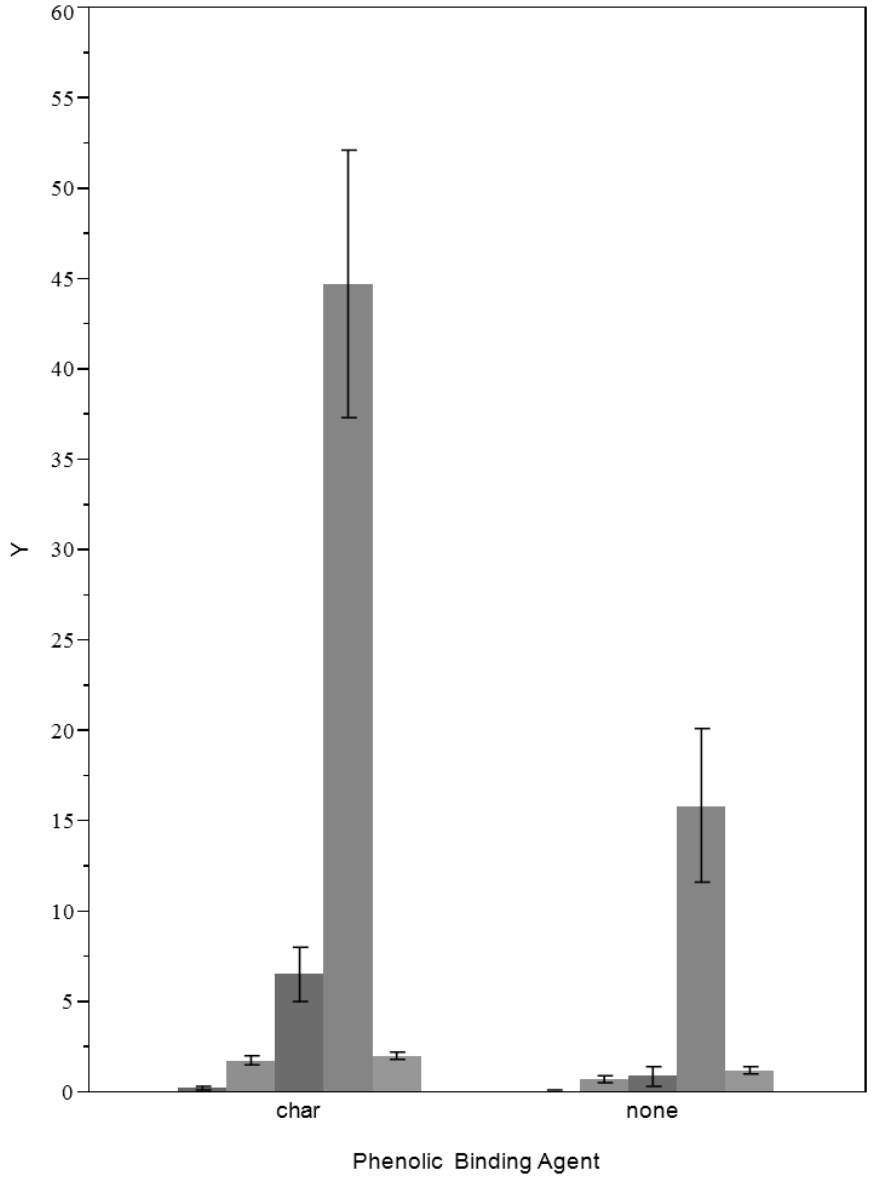
PBA	*in vitro* IBA conc (µM)	Plants producing roots *in vitro*[1]	*in vitro* Root number[1]	*in vitro* Root length (mm)[1]	Plants producing roots *ex vitro*[1]	*ex vitro* root number[1]	*ex vitro* root length (mm)[1]	*ex vitro* lateral root number[1]	*ex vitro* leaf number[1]
0g/	0	0.0±0.0	0.0±0.0	0.0±0.0	1.80±0.54	0.68±0.26	5.52±2.36	0.0±0.00	0.72±0.20
L	5	0.33±0.21	0.07±0.04	1.27±0.80	3.00±0.58	1.23±0.27	26.8±4.72	1.43±0.54	1.73±0.21
AC	10	0.0±0.0	0.00±0.0	0.00±0.0	2.50±0.56	1.13±0.41	12.17±3.94	0.30±0.30	1.27±0.27
	20	0.17±0.17	0.03±0.03	0.50±0.50	2.33±0.33	0.77±0.08	17.1±3.42	1.60±0.52	1.10±0.07
1g/	0	0.0±0.0	0.16±0.07	5.08±2.36	3.40±0.75	1.44±0.33	47.4±11.58	5.84±1.75	1.76±0.30
L	5	0.83±0.54	0.20±0.12	4.20±2.72	4.00±0.26	2.30±0.24	51.1±9.85	9.10±2.33	2.37±0.27
AC	10	0.83±0.65	0.30±0.23	3.60±2.29	3.67±0.21	1.57±0.35	38.0±10.94	4.87±2.22	1.97±0.25
	20	0.83±0.07	0.20±0.07	5.23±1.88	3.50±0.56	1.63±0.29	42.7±7.51	6.00±1.27	1.87±0.22
Significance									
AC		*	*	**	**	**	**	**	**
IBA conc.		NS	NS	NS	NS	NS	NS	NS	*
IBA conc. x AC		NS	NS	NS	NS	NS	NS	NS	NS

[1]Values represent means ± SE.
[2]NS, *, **: Nonsignificant or significant at p=0.05 and 0.01, respectively. IBA=Indole butyric acid. AC=Activated Charcoal.

Figure 3.1. Comparison of explants after 8 weeks of culture in media with 5 different basal salt compositions. From left to right: MS, 1/2 MS, WPM, DKW, and Blaydes.

Figure 3.2. Comparison of plantlets *in vitro* after 8 weeks of culture. MS media with AC (left) and MS media without AC (right).

Figure 3.3. Growth responses to in vitro and ex vitro culture as grouped by phenolic binding agent treatment.



Each error bar is constructed using a 95% confidence interval of the mean.

Figure 3.4. *in vitro* rooting and growth of plants at 6 weeks after treatment with (left) 5 µm IBA; and (right) 5 µm IBA plus AC. Across all treatments rooting was more frequent and plants were more robust when AC was incorporated *in vitro*.

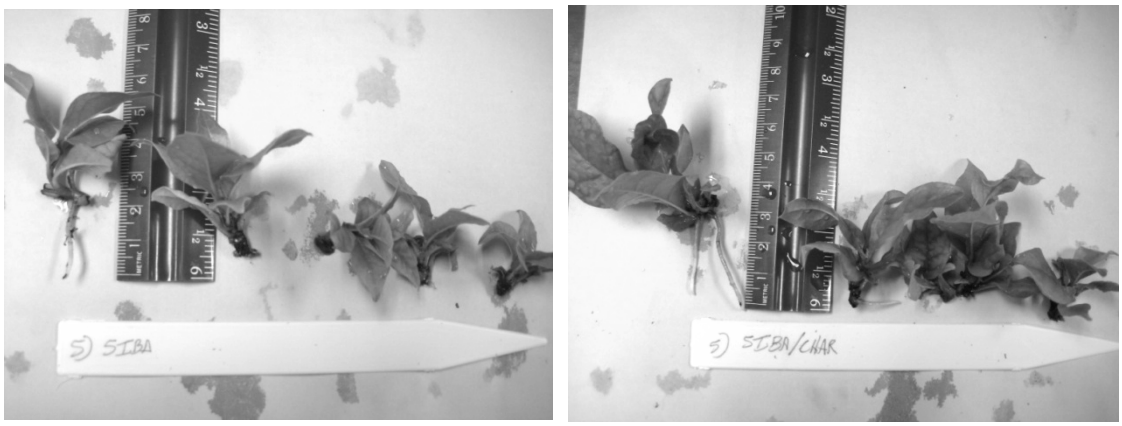
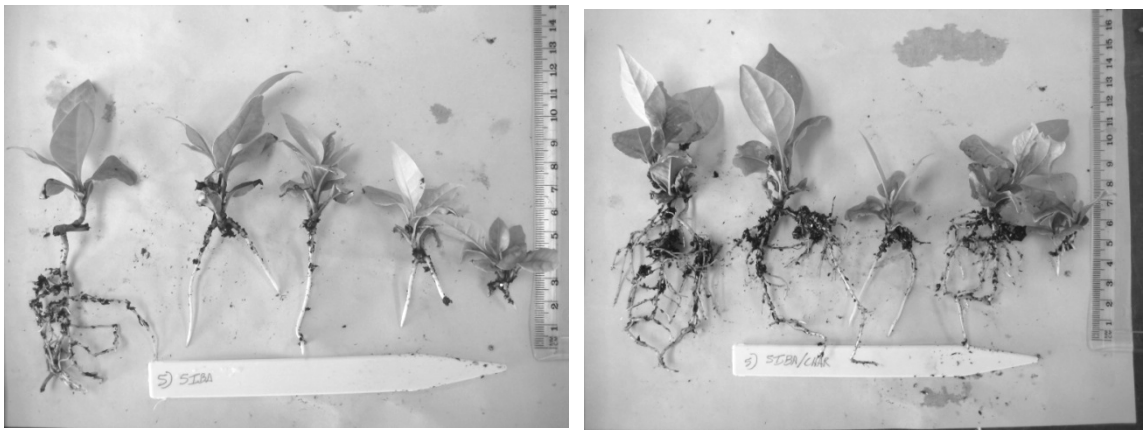


Figure 3.5. *ex vitro* rooting and growth of plants at 6 weeks after treatment *in vitro* with (left) 5 µm IBA; and (right) 5 µm IBA plus AC. When AC was incorporated *in vitro*, across all treatments, roots were more frequent, more lateral roots were present, and more leaves were produced *ex vitro*.



CHAPTER FOUR
CONCLUSIONS AND FUTURE RESEARCH

A wealth of scientific knowledge and technical application of that knowledge is required to be a breeder and propagator of plants. In Genus Magnolia; chromosome number, genome size, and overall diversity must be considered for limitations of reproductive compatibility in hybridization efforts to be fully understood. These studies have shown for the first time within the full range of ploidy levels of this genus, ranging from 2n=2*x*=38 to 2n=8*x*=152, that there is a correlation between genome size and chromosome number. Concordance of chromosome number with genome size supports hypotheses that numerical mutations are a major factor in evolutionary divergence (Ranney, 2006).

There is long history of successful interspecific hybridization in magnolia dating to the nineteenth century. The surveyed accessions documented an extensive list of species and selections from numerous interspecific hybrids. The data we have gathered allowed us to validate hybridity in interploid crosses and intersectional crosses where species significantly vary in relative genome size and use this information for designing breeding schemes.

Sterility reported in several accessions was associated with the relative genome size and chromosome number indicating frequently an aneuploid condition is the potential outcome of interploid hybridization. This research has also shown that partial fertility can be retained in aneuploid taxa when the chromosome number is 2n=5x=95 or

higher, probably due to the formation of unreduced gametes or lack of chromosomes tolerated by larger genomes.

No triploid taxa (2n=3x=57) surveyed in this study have been documented as parents in a successful cross. Numerous triploid hybrid cultivars with ornamental merit exist, yet they represent “a dead end” in a breeding program due to infertility. When recombination of favorable traits is desired beyond the F1 generation, retention of reproductive capability is essential. Based on the chromosomal number of a species or hybrid, as inferred from relative genome size, predictions can be made as to better fertility outcome in crosses between species of varying ploidy level. Taxonomic alignment based on the molecular phylogeny models can support this effort.

During the course of these studies, preliminary breeding work was initiated to gain further insight into the reproductive behavior of various magnolia species. Breeding schemes were selected based on this study of relative genome size and polyploidy, as well as the taxonomic models that place *Michelia* and *Manglietia* within Genus *Magnolia*. Gleaning from the recent successes of breeders Dennis Ledvina; Greenbay, WI, and Bill Smith; Richmond, VA documented by Figlar (2011), work was first focused on the species *M. insignis* because the red coloration in the tepals is a desirable trait to introgress into potential hybrids. Breeding efforts were also initiated in Subgenus Yulania, focusing on the unexplored genetic diversity within Section Michelia. Several of the selected species had never been used before in the specific cross combinations. Using the obtained information on genomic variability in conjunction with an objective of combining desirable traits, over the past three years the following new, interspecific,

intersectional*, and interploid^ crosses have been successfully performed within Genus Magnolia:

Subgenus Magnolia

M. insignis (MGA 355) × *M. grandiflora* 'Kay Parris' (SCC specimen)*^

M. insignis (MGA 355) × *M. fraseri* (pollen harvested from West Virginia)*

Subgenus Yulania

M. stellata 'Royal Star' (SCC specimen) × *M. figo* var. *skinneriana* (Parris)*

M. foveolata 'Shibamichi'(MGA 356) × *M. laevifolia* 'Gail's Favorite' (NCSU 2008-268)

These successful crosses provide evidence which supports our conclusion that viable hybrids can be obtained from careful consideration given to genome size, ploidy level, and recent taxonomic alignments. These hybrid plants will be evaluated for ornamental qualities, and will ultimately provide insight into reproductive biology, inheritance of traits, and asexual propagation protocols. Certainly these new hybrids will provide research opportunities for years to come.

With a vastly improved understanding of the role that taxonomic relationships and polyploidy play in the sexual reproduction of magnolia, a better understanding of how asexual propagation may be used to facilitate the development and mass production of new cultivars is essential. As such, asexual propagation, particularly *in vitro* methods are likely to be necessary to create a platform for chromosome doubling and fertility restoration. Specifically, sequential incorporation of cytokinins, auxins, and phenolic binding agents to condition explants for doubling efforts, and *ex vitro* establishment.

Preliminary studies have been initiated to examine the effects of liquid media culture during the micropropagation process. Initial results indicate that liquid media culture may prove to be a more efficient process and allow for better dissipation of phenolic substances from the explant tissue, while maintaining comparable multiplication and establishment results to traditional semi-solid media preparations.

Thus future work with Genus Magnolia should proceed on several fronts to ensure the rich diversity it contains is preserved and developed for future generations:

- *In situ* and *ex situ* conservation of species, particularly endangered species endemic to South America and Asia. This is crucial to ensure access to these species with unknown genetic potential.
- DNA analysis of populations to discern genetic diversity within and between species.
- Hybridization and selection to continue development of cultivars for enjoyment in gardens around the world.
- Interspecific germplasm preservation for introgression of improved environmental tolerances.
- Refinement of asexual propagation techniques, namely micropropagation, to ensure rapid propagation of rare, endangered, and noteworthy taxa for ongoing conservation, research, breeding, and the appreciation of the gardening public.

Magnolia, as a basal angiosperm, has a rich evolutionary history. Through novel approaches we can begin to facilitate knowledge about the genus to better understand evolutionary relationships and adaptation to changing environmental conditions to stimulate efficient preservation and utilization of Magnolia species.

APPENDICES

Appendix A

REFLECTIVE SUMMARY OF WORK

The collection and testing of so many samples may have turned into a painfully repetitive exercise if it were not for the immense respect I have developed for the individuals that brought these magnolia species into cultivation and escorted scores of hybrids into existence. Without their work, this study would have never been possible. My background reading that lead to the development of Chapter One allowed me to visualize each sample I held as a piece of living history. Nevertheless, I was using modern technology to measure the relative weight of the genetic material present in the average cell of each named plant, thus turning the product of a plant explorer or breeder's work into a number derived from a mathematical equation. Having adventured with friends from the Magnolia Society International to some of the world's finest assemblies of Magnolia, I recognize these plants are not the outcome of equations. They are the results of nature, results of hope and frustration, anticipation and tenacity, passion and heartache. But the numbers have consequence, and by better understanding them, greater hope with less heartache may be realized.

For me, this study has painted an abstract picture of the dance that takes place when gametes from Magnolias meet. Within species the match is so perfect that little or no genetic information is lost or gained that would lead to significant variation in genome size. The partners are well acquainted, the dance is well rehearsed, no toes are stepped on, and the performance is flawless. Yet, we have learned that Magnolia species may

dance with different partners. Though they may have been separated by mountains, plains, or oceans, and eons of time, there is an affinity that still exists. The harmony of the genetic sequences ring like a musical composition. Therefore, the tune is familiar, and though the partners may stumble, the jubilation of the reunion often shines through in the dance.

The process of discovery highlighted in the micropropagation study was equally rewarding. Having been a professional plant propagator for nearly a decade I believed I had a comfortable knowledge of the personality of plants and how they respond to the stress of being made less than whole. I thought that I understood enough about how to manipulate the environment around them to ensure that totipotency could be realized. I now know that to be a conventional plant propagator one only has to be the manager of a 4 piece rock n' roll garage band. Once the singer, guitarist, bassist, and drummer know their parts everything falls into place and the parts become whole because the music is inherent to them. Once the bassist finds the rhythm, chords emerge from the guitar, and percussion is soon enhanced by vocals. Similarly, roots emerge from pericycle tissue in a hardwood cutting largely because of the auxin inherent to the substantial tissue harvested from the stock plant. Root growth stimulates cytokinin production striking a chord with shoots and leaves that rhythmically restore the ratio of these hormones, and ultimately the whole plant sings through its growth and development. The garage may not be all that clean, but the musicians are happy to play anywhere because they already hardened to a rugged lifestyle. The mist bench or nursery pad is adequate for the plant to become whole because it is likely in a more favorable environment than where it originated.

Conversely, *in vitro* propagation is more like attempting to be the conductor of a philharmonic orchestra in the kitchen while extended family is visiting at Thanksgiving. The volume of the music is similar to the rock band, but there are hundreds of musicians and instruments to synchronize. The delivery of cues from the conductor must be precise and the musicians are more selective of the venue in which they perform. The tissue collected from the stock plant is less replete with nutrient reserves and natural hormone content. It requires immediate delivery of these nutrients and hormones to sustain the tissue and later direct the focus of cellular growth. Due to interactions between these substances, their incorporation is sometimes sequential. In the philharmonic, the musicians do not all play continually. There is a place for the trumpet to blare and the timpani to resonate but they can't simultaneously dominate the sound or the performance will come to an unharmonious conclusion. Despite sequential supplementation of necessary ingredients, the closed environment provided to protect the tissues in the sucrose saturated media stifles the dispersal of metabolic wastes from the developing explants. The instruments in a superbly performed symphony still generate reflected sound and delayed echoes that would distort the performance if sound dampening curtains and acoustic tiles were not properly placed in the concert hall. Phenolic binding agents such as activated charcoal act like acoustic tiles and absorb the "noise" in the micropropagation vessel. If the conductor and propagator are wise, skillful, and diligent, the pieces become whole and their work gains a life of its own. We need to be able to read music, sing, dance, and play an instrument simultaneously. If plants are developed with the inspiration of sound science and a creative eye, they will grace gardens beyond

the life of the breeder, just like the melody of a classic song can transcend generations. May the song and dance continue.

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